EVALUATION OF FIBER DEGRADING ENZYMES IN COTTON STALK BASED PELLETED COMPLETE FEED OF GROWING GOATS

T H E S I S

Submitted

In partial fulfillment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY

IN

ANIMAL NUTRITION

BY

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DECLARATION OF STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled **"EVALUATION OF FIBER DEGRADING ENZYMES IN COTTON STALK BASED PELLETED COMPLETE FEED OF GROWING GOATS"** or part thereof has not been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis/publication of any University or scientific organization. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged.

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Place: Nagpur
Date: / / 2023

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This is to certify that the thesis entitled, **"EVALUATION OF FIBER DEGRADING ENZYMES IN COTTON STALK BASED PELLETED COMPLETE FEED OF GROWING GOATS"** submitted by Shri **NIPANE SURESH FATTUJI** to the Maharashtra Animal and Fishery Sciences University in partial fulfillment of the requirement for the degree of **Doctor of Philosophy** has been approved by the Student's Advisory Committee after examination in collaboration with the External Examiner.

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Date: / / 2023 **Suresh Fattuji Nipane**

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ABBREVIATIONS

Abbreviations		Full Form
&	:	And
ADF	:	Acid Detergent Fiber
ADG	:	Average Daily Gain
AOAC	:	Association of Official Analytical Chemicals
AVG	:	Average
b.wt.	:	Body Weight
CF	:	Crude Fiber
CS	:	Cotton Stalk
CP	:	Crude Protein
DF	:	Degree of Freedom
DM	:	Dry Matter
dl	:	Deciliter
d	:	Day
DMI	:	Dry Matter Intake
EE	:	Ether Extract
EFE	:	Exogenous Fibrolytic Enzyme
et. al.	:	Ethically All
FCE	:	Feed Conversion Efficiency
FCR	:	Feed Conversion Ratio
Fig.	:	Figure
g	:	Gram
h	:	Hour
HC	:	Hemicellulose
IVDMD	:	*In vitro* dry matter digestibility
IVNDFD	:	*In vitro* neutral detergent fiber digestibility
IVADFD	:	*In vitro* acid detergent fiber digestibility
kg	:	Kilogram
Mcal	:	Mega Calorie
mEq/L	:	Milli Equivalent per liter
g/d	:	Gram Per Day

mg	:	Milli Gram
ml	:	Milliliter
MSS	:	Mean sum of Squares
NDF	:	Neutral Detergent Fibre
NFE	:	Nitrogen Free Extract
NH_3-N	:	Ammonia Nitrogen
No.	:	Number
NS	:	Non-Significant
NPN	:	Non-Protein Nitrogen
OM	:	Organic Matter
pH	:	Hydrogen Ion Concentration
/	:	Per
%	:	Percent
Rs.	:	Rupees
*	:	Significant
<	:	Less Than
>	:	Greater Than
P<0.01	:	Significant at 1% Level
P<0.05	:	Significant at 5% Level
SE	:	Standard Error
SRL	:	Strained Rumen Liquor
SS	:	Sum of Squares
TCA-ppt-N	:	Tri-chloro Acetic Acid-Precipitate-Nitrogen
TDN	:	Total Digestible Nutrient
TMR	:	Total Mixed Ration
TVFA	:	Total Volatile Fatty Acids
Viz.	:	Videlicet Namely

INTRODUCTION

Worldwide demand for animal origin foods (milk, meat and eggs) is increasing tremendously in the recent years and will continue to increase in the near future. More so in the Asia, where the demands for livestock products are anticipated to outstrip the current production levels, and the demands are projected to grow by two to three fold by 2050 (Devendra and Leng, 2011). To achieve these targeted levels of production, efficient feeding of livestock is necessary as feed is the major driver of livestock production.

Goat is important livestock species for the nourishment of rural poor people in India and other countries. Goat raring plays important role in providing subsidiary income and livelihood to millions of landless laborers, marginal farmers, and small farmers. As per 20th Livestock Census (2019), the goat population of India is 148.88 million which constitute 27.74% of the total livestock population and contributes about 3% of total milk production and 14.25% of total meat production in the country.

Goats mostly thrive on grazing of natural grasses as well as browsing on shrubs and various tree leaves. These feed resources are characterized by low digestibility, low ME, and low CP besides poor availability of minerals and vitamins. Moreover, there is a scarcity of grazing land due to the ever-growing human population, industrialization, deforestation, etc. Hence there is a need to adopt scientific feeding of goat and making utilization of various non-conventional feed resources for reducing the unit cost of production.

The source of green fodder in India is from cultivation of agricultural land, forest and fallow lands. Currently, the area available for fodder cultivation is decreasing, emphasizing the need for efficient utilization of available feed and fodder resources. There is a deficiency of 44% for concentrate feed ingredients, 35.6% for green fodders, and 10.95% for dry roughages in India (IGFRI, 2015).

Crop residues are bulky and contain more than 18 percent crude fiber. Crop residues comprise straws and stovers obtained after harvesting the crops.

These crop residues have for a long time been a major source of food for animals and will continue to be so for the foreseeable future. Though crop residues are the largest feed resource in the country, their use and development has not received proper recognition due to their bulkiness, poor nutrient density and high transport costs. This importance of crop residues will continue to grow due to a deficit in feed resources and a rising demand for livestock feed, which cannot be fulfilled by green fodder alone. The crop residues are usually consumed by large ruminants but small ruminants like goats are reluctant to consume them. Several attempts have been made by the researchers to improve the nutritive value, digestibility, and utilization of low-cost feed by various processing methods.

Out of the total crop residues produced in India every year, some are utilized as fodder for the animals and most of them are burnt as such to remove waste in land or used as fuel and for composting. Burning crop residues in general, and cotton stalks in particular, is laborious, time-consuming and expensive. Combustion also leads to emissions of greenhouse gases such as CO_2, NO_2, SO_2, NH_3 and volatile organic compounds. Existing pollutants from vehicles and industrial pollution affect air quality and visibility in urban areas. Ultimately lead to global warming and climate change (IARI, 2012). Heat generated due to burning kills soil micro-organisms and eco-friendly insects too. So, combustion leads to loss of valuable soil organic matter and nutrients. Hence, proper utilization of crop residues can overcome the fodder shortage due to increase in livestock population. The best way to efficiently utilize the crop residues is to prepare complete feeds from crop residues and concentrates mixture. Major crop residues produced from different crops are maize stover, sorghum straw, wheat straw, rice straw and gram straw etc.

India is one of the world's largest cotton producers, accounting for approximately 26% of the world's cotton production (https://cotcorp.org.in). Cotton accounts for nearly 40% of global fiber production. Among the eighty cotton producing countries, half of the world's cotton is produced by U.S., China, and India alone. Availability of cotton stalk in India accounts for 22.33 million tons cultivated in 9.175 million hector areas and in Maharashtra 6.24 million tons

of cotton stalk is available from 3.124 million cultivated hectors area. Since its use in Vidarbha region of Maharashtra is restricted as biomass-fuel only, the conversion of cotton stalks as animal feed can therefore lower the feeding cost of livestock and ultimately bring economic relief for the farmers in stress. The amelioration of cotton stalks as feed will improve animal nourishment (Vinjamur *et al.,* 2015). Cotton crop wastes usually referred as cotton stalk consist of the main stem, branches, bur, boll rinds, bracts, peduncle, roots, petioles, and leaf blades left as residual biomass after harvesting the floral cotton bolls. It is roughly 3–5 times the weight of the produced cotton. Cotton stalks include approximately 46 percent alpha cellulose and approximately 26 percent lignin. The cotton stalks are low in protein, energy and have poor digestibility, therefore efforts are necessary to improve its quality as roughage for ruminants.

After harvesting the cotton crop, the remaining biomass is highly rich in cellulose, hemicelluloses which are the crucial source of nutrients for the ruminant animals (Patil *et al.,* 2007). The cotton plant residue (cotton straw) comprises of stalks and estimated nearly 2.5 to 3.5 tons of cotton stalks per acre. Such harvested cotton crop by-products can be useful as new non-conventional roughage resource in animal feeding (Sharma-Shivappa and Chen, 2008). The primary challenge for use of cotton stalk biomass is to break complex ligno-cellulosic structure facilitating the rumen enzymes leading to ruminal microbial degradation (Travaini *et al.,* 2016). Cellulosic waste is generated maximum from the agricultural sector. Cellulose accounts for up to 50% of total weight (dry) of plant. Agricultural residues are rich in cellulosic materials, which are renewable, cheap, and mostly inexpensive resources.

Now a day, ligno-cellulosic wastes appears to be important for ruminants (Sadhu and Maiti, 2013). Lignocellulose is composed of cellulose (35%–50%), hemicellulose (15%–30%), pectin (2%–5%), and lignin (12%–35%). Cellulose and hemicellulose are primarily composed of C5 and C6 sugars. These C5 and C6 sugars have potential to convert into methanol, ethanol, and lactic acid (Ragauskas *et al.,* 2014).

Enzymes are naturally occurring and produced by all living organisms. Enzymes are proteins which are consisting of a chain of amino acids. They catalyze reactions by binding to substrates and stabilize the entire reaction process up to product formation (Sheppy, 2001). Activity of enzyme depends on its type, the species of animal used, pH, temperature and gastro-intestinal conditions, dosage, substrate, degradation of the exogenous enzyme along the tract, practical management conditions and other factors (Rojo *et al.,* 2007). Enzymatic feed products for ruminants are concentrated fermentation products with specific enzymatic activity, but the main activity is usually controlled. Ruminant enzyme preparations are evaluated for their ability to degrade plant cell walls (Pendleton, 1998). These enzymes fall into general classification of cellulases and xylanases.

Cellulase is an important enzyme fraction utilized by several industrial degradation processes involving biomass feedstock for assisting in production of valuable products. Cellulases account for about 8% of planet-wide enzyme demand (Bon and Ferrara, 2013). Cellulase production leads to solid-state fermentation because of cellulose producing microorganisms (*Trichoderma sp.*, *Aspergillus sp.,* etc.). The cellulases and xylanases can vary widely between products and differences in the relative proportions and activities of these individual enzymes affect the effectiveness of these products for cell wall degradation. (Beauchemin *et al.,* 2001). Xylanases and cellulases have been most commonly used for ruminants, although there have been assessments with ferulic acid esterase, proteases, phytases, and amylases to break ferulic acid bridges, attack cell wall nitrogen-containing compounds, increase phosphorus absorption, and improve starch digestion, respectively (Beauchemin and Holtshausen, 2010; Arce-Cervantes *et al.,* 2013).

Commercial cellulase production principally implements the techniques of solid-state or submerged fermentation strategies in fed-batch. The commercial cellulase production uses the technique of fermentation as the conversion/degradation of complex organic substrates into simpler molecules by action of microorganisms. Substrate costs and costs involved in fermentation

process design are the main reasons behind the increasing cost of the enzyme in the market (Khan and Singh, 2011).

The liquid enzymes are generally less stable than solids although they may have greater activity than their solid counterparts. In general practice, loss in activity of liquid enzymes occurs due to thermal and mechanical treatments (Mascarell and Ryan, 1997). Methods must be in place to determine whether the required amount of enzyme has been added to the feed and whether the additive has been distributed adequately in the feed.

Fibrolytic enzymes, which were previously used mainly for non-ruminants (Graham and Balnave, 1995), seem also to be effective in ruminants (Krause *et al.,* 1998; Lewis *et al.,* 1999; Rode *et al.,* 1999). The use of exogenous fibrolytic enzymes holds great potential for efficient forage utilization and improving the productive ability of ruminants. The exogenous fibrolytic enzymes used in ruminants are from fungal (*Trichoderma longibrachiatum*, *Aspergillus niger*, and *A. oryzae*) and bacterial (*Bacillus spp*., *Penicillium funiculosum*) sources with high cellulosic and hemicellulosic activity, and are incorporated in liquid or granular form with the total mixed ration, hay, silages, concentrates, supplements or premix, and increase the availability of nutrients (Beauchemin *et al.,* 2004).

Hence considering the abundant availability of cotton stalk in the region, the present study was conducted to utilize waste cotton stalk by subjecting it with liquid fibrolytic enzymes treatment and its incorporation in pelleted complete feed of growing goats with the following objectives:

OBJECTIVES

1. To study the assay of different fiber degrading enzymes.
2. Standardization of dose and duration of treatment of fiber degrading enzymes in the shredded cotton stalk.
3. To study the *in vitro* dry matter digestibility of enzyme treated cotton stalk in goats.
4. To study the effect of supplementation of enzymes in cotton stalk based pelleted complete feed in growing goats.

5. To study the economics of supplementation of fiber degrading enzymes in the cotton stalk based pelleted complete feed of growing goats.

REVIEW OF LITERATURE

Studies on the stall-feeding system is gaining importance, as the current scenario predicts a continued decline in natural grazing. Complete feeding system is a better option than feeding concentrates and roughage separately or grazing with supplemental feed to goats. Complete feeding systems is one of the option to exploit the potential of animal feed resources in the best possible way. The benefits of complete feeding include providing goats with a balanced diet and better utilization of locally available feed resources and reduced feed cost and labour. Complete feed with use of fibrous crop residues is a noble way to increase voluntary intake and thus animal's production performance at comparatively cheaper cost. Feeding complete diet also stabilizes ruminal fermentation and thereby improves nutrients utilization.

The cotton stalks available in field after the last picking of cotton is about 1.85: 1 of cotton seed (Hunsigi and Krishna, 1998). The cotton stalks as such is unpalatable and has poor nutritive value due to highly lignified cell wall contents. Earlier experiments indicated that cotton stalk could be made palatable and its nutritive value could be improved by incorporating in complete diets as a roughage source after processing with expander extruder in calves (Kirubanath *et al.,* 2003) and lactating animals (Nagalakshmi *et al*., 2004). Thus, with proper treatment huge quantity of cotton stalks can be made available for livestock feeding which is presently used as fuel or organic manure.

Pelleting of complete feed is produced by compacting and pressing mixes of all feed ingredients through the opening of the die using mechanical process. Pelleting low quality forage-based feed improves consistency, increases bulk density, simplifies handling and reduces waste. All of these contribute to better animal nutrition (Stevens, 1981). Pelletized feed is an accurate blend of all feed ingredients that are thoroughly mixed to avoid separation and selection. The idea of a pelleted complete feed system using locally available agricultural residues thus seems better option.

The literature on pertinent components of the current investigation was thoroughly screened. The relevant and related findings of various scientists' research work are provided below.

2.1 Chemical composition of cotton stalk

Kirubanath *et al.* (2003) investigated on effect of processing cotton straw based complete diet with expander-extruder on performance of crossbred calves. The chemical composition of cotton stalk was reported to be 97.68% DM, 95.59 % OM, 6.63% CP, 2.99% ether extract (EE), 76.32% NDF, 67.47% ADF, 18.57% cellulose and 48.50% lignin on dry matter basis.

Nagalakshmi and Reddy (2011) evaluated effect of feeding expander extruder processed complete diet (EEP) containing cotton stalk (CS) as sole roughages source on growth performance and milk production. In their study the nutrient composition of cotton stalk was observed to be 92.89% DM, 7.02% CP, 37.08% CF, 1.02% EE, 49.71% NFE, 4.37% TA, 75.80% NDF, 62.51% ADF, 13.29% hemicellulose and 11.58% cellulose.

Hashim *et al.* (2017) determined comparative analysis of chemical composition and *in vitro* digestibility of Bt versus non-Bt cotton crop residues. Chemical composition of Bt and non-BtCCR of tender branches were found to be 92.18, 90.7% DM, 6.49, 5.50% Ash, 7.29, 6.09% CP, 43.30, 64.20% CF, respectively.

Jadhav (2019) conducted research on utilization of cotton stalk in complete pelleted feed of growing goats. The chemical composition of untreated cotton stalk was found to be DM 96.83%, CP 3%, EE 1%, CF% 51.5%, TA 4%, NFE 40.5%, while fiber fraction of untreated cotton stalk NDF 82.6%, ADF 71.4%, cellulose 17.7%, and hemicellulose 11.2% and lignin 56.00%.

Ayaşan *et al.* (2020) studied the nutrient contents and *in vitro* true digestibility (IVTD) of parts of matured and harvested cotton plant 140 days after planting. Four replicate plants were separated into nine parts, namely lower stalk, central stalk, upper stalk, corn ear stalk, corn ear shuck, kernals, corn cob, leaf,

tassel, plus the entire plant. The samples were dried and ground for analysis. Significant differences in nutrient content were observed among parts of the corn plant. The highest crude protein (CP) content was found in the leaf (12.41%), followed by the grain (12.37%). Dry matter (DM) varied from 91.25% to 96.07%. The highest ether extract (EE) was in the grain (2.84%), and the upper stalk contained the least EE (0.29%). The parts also differed in their contents of crude cellulose (CS) and crude ash (CA) ($p<0.001$). Most organic matter (OM) was found in the corn cup (94.27%).

Burghate (2021) studied on replacement of gram straw with ozone treated cotton stalk supplemented with yeast and multienzymes in pelleted complete feed of growing goats. Proximate and fiber composition of cotton stalk in her study was found to be DM% 95.46, CP% 3.12, EE% 1.06, CF% 52.46, TA% 7.56, NFE% 35.80, NDF% 61.46, ADF% 45.72, hemicellulose% 15.74 and cellulose% 19.84.

2.2 Chemical composition of treated cotton stalk

Ben-Ghedalia *et al.* (1980) observed the effect of ozone and ammonium hydroxide treatments on the composition and *in-vitro* digestibility of cotton straw. They reported that, ammonium hydroxide treatment did not affect the lignin content, but a reduction of 50% in lignin and hemicellulose, and a corresponding increase in cell contents, was found in the ozone-treated materials. As a result, composition of cellulose and hemicellulose of untreated cotton straw, ozone and ammonium hydroxide treated cotton straw were found as 40.8%, 39.7%, 40.04% and 10.6%, 5.45%, 5.30%, respectively.

Miron and Ben-Ghedalia (1981) studied the effect of chemical treatments on the degradability of cotton straw by rumen microorganisms and by fungal cellulase. Three different chemical treatments-sulfur dioxide, ozone, and sodium hydroxide were applied on cotton straw, and the effect on cell-wall degradability was assessed by using rumen microorganisms and *Trichoderma reesei* cellulase. Percent composition of untreated cotton straw was 11.2 ± 0.46, 35.6 ± 0.29, 18.6 ± 0.25 hemicellulose, cellulose and lignin, respectively. Sulfur dioxide

(applied at 70^0 C for 72 hr) did not change the lignin content of cotton straw but reduced the concentration of hemicellulose by 48%. Ozone exerted a dual effect, both on lignin (40% reduction) and hemicellulose (54% decrease). The treatment with NaOH did not solubilize cell-wall components.

Reddy *et al.* (1992) evaluated effect of physical processing on nutritive value of cotton straw in goats and sheep. Cotton straw subjected to physical processing, grinding and pelleting and chemical composition; processed cotton straw was comparable to a poor quality roughage. Chemical composition of ground and pelleted cotton straw/stalk was found similar in both physical processing methods. On dry matter basis, value of CP% (4.23, 4.17), CF% (42.66, 39.60), EE% (1.66, 1.36), NFE% (46.50, 49.23), TA% (4.95, 5.64), NDF% (60.76, 60.93), ADF% (46.46, 46.62), cellulose% (39.28, 39.68), hemicellulose% (14.30, 14.31) and lignin% (6.17, 6.27) was found in grinding and pelleting cotton stalk, respectively.

Grewel *et al.* (2003) studied on effect of cotton stems with addition of urea and formic acid. Ground cotton stems (T_2 and T_3) and stems treated with 0.5% urea (T_4 and T_5) were ensiled with pearl millet green fodder in double lined plastic bags of 3 kg capacity for 50 days. Formic acid (0.4% v/v) was sprayed on T_3 and T_5 silages. The treatments were compared with pearl millet silage alone (T_1) which constituted the control. The chemical composition of cotton stem and urea treated cotton stem where found to be 90%, 40% DM, 95.82%, 95.80% OM, 6.10%, 7.44% CP, 57.2%, 58.20% CF, 1.16%, 1.05% EE, 4.18%, 4.20% Ash, 31.28%, 29.11% NFE, 39.71% 38.95% cellulose, 78.42%, 79.26% NDF, 63.12%, 65.85% ADF, 16.57% 18.66% ADL and 15.30%, 13.41% hemicellulose on dry matter basis, respectively.

Hamza *et al.* (2005) carried out research to study the effect of incubated cotton stalks and rice straw with white rot fungi growth (*Pleurotus ostreatus*) on the digestibility (*in vitro* and *in vivo*) and feeding value of cotton stalks and rice straw. Chemical composition and fiber fraction of *Pleurotus ostreatus* treated cotton stalks and untreated cotton stalks showed DM (89.4, 89.20%), OM (89.18, 86.20%), CP (4.94, 11.10%), CF (44.13, 23.06%). EE (1.36, 1.02%), NFE (39.75,

51.02%), ASH (10.82, 13.80%), NDF (92.40, 72.90%), ADF (75.60, 59.70%), ADL (33.90, 26.30%), cellulose (41.7, 33.4%) and hemicellulose (16.80, 13.20%) content, respectively.

Kaur and Phutela (2018) studied on morphological and structural changes in paddy straw influenced by alkali and microbial pre-treatment. Chemical composition of rice straw treated with microbes (*Plerotus florida*) and untreated rice straw was found to be 69.1%, 74.5% NDF, 50.6%, 51.5% ADF, 5.6%, 8.2% ADL, 49.5%, 45.5% cellulose and 19.5%, 23.0% hemicellulose on dry matter basis, respectively.

Muruz *et al.* (2019) determined the effects of a mixed bacterial inoculant possessing ferulic acid esterase (FAE) activity and exogenous fibrolytic enzyme (EFEs) products on the chemical composition, conservation characteristics and digestibility of corn stover. The corn stover was treated with deionized water (control), EFEs (10 U cellulase + 60 U xylanase units/g of substrate dry matter) or EFEs + FAE inoculant (FAEI) ($1.3x10^5$cfu/g fresh forage). The chemical composition of corn stover treated with deionised water (control), EFEs and EFE_S + FAE were NDF (69.65, 64.86, 65.04%), ADF (46.28, 42.92, 43.15%), ADL (12.10, 12.24, 12.16%) and Ash (9.20, 9.33, 9.45%), respectively.

Shembekar (2019) studied the effect of utilization of ozone treated cotton stalk in pelleted complete feed of eighteen growing goats (4 to 6 months of age) for 90 days. The goats were randomly allotted to three dietary treatments as T_0, receiving pelleted complete feed made from 60% gram straw as roughage and 40% concentrates, T_1 receiving pelleted feed containing 15% ozone treated cotton stalk (OTCS) and 45% gram straw as roughages along with 40% concentrates while, T_2 group received pelleted complete feed containing 30% OTCS and 30% gram straw and 40% concentrates. The roughage to concentrate ratio was maintained as 60:40 in each group. Chemical composition (% DM basis) of ozone treated cotton stalk was found to be DM% 96.60, OM% 97.00, CP% 3.5, EE% 1.3, CF% 51., TA% 3, NFE% 41.20, while fiber fraction composition NDF% 89.9, ADF% 70.3 and hemicellulose% 19.6, cellulose % 20.3 and lignin % 46.

Balakesava Reddy *et al*. (2020) conducted a trial to evaluate paddy straw treated with maize spent liquor (MSL) at varying levels and different days of incubation for *in vitro* digestibility of nutrients. Data revealed that chemical composition of paddy straw was 90.33% (DM), 85.60% (OM), 3.75% (CP), 1.54% (EE), 36.92% (CF), 14.40% (TA), 43.39% (NFE), 76.68% (NDF), 49.19% (ADF), 6.42% (ADL), 27.48% hemicellulose and 34.68% cellulose.

Ma *et al*. (2020) explored the effects of ammoniation treatment on the chemical composition and *in vitro* digestibility of rice straw in Chinese Holsteins. Rice straw was stored in polyethylene bags (35 × 25 cm, 350 g per bag) including (i) no additives (RS); (ii) 5% urea (5U, dry matter (DM) basis); (iii) 9% corn steep liquor + 5% urea (9C5U, DM basis); (iv) 9C2.5U; and (v) 9C2.5U + 3% molasses (9C2.5U3M, DM basis). The air-dry matter of the mixture was kept at the same level at 55% for all treatments. Fifteen bags (5 treatments × 3 replicate) were prepared and stored at ambient temperature (25 ± 3^0 C). The chemical composition of rice straw after 60 days was found DM (95.96, 95.87, 95.69, 95.86 and 95.96%), CP (5.79, 9.27, 11.38, 11.02 and 9.51%), Ash (13.91, 12.94, 13.32, 14.20 and 13.18%), NDF (65.41, 65.30, 32.02, 64.07 and 62.37%), ADF (54.64, 53.19, 47.84, 48.90, and 49.72%) and ADL (1.30, 1.35, 1.22, 1.36 and 1.30%) for RS, 5U, 9C5U, 9C2.5U and 9C2.5U3M, respectively.

Amido *et al.* (2021) studied the potentiality of the composting process and biological pretreatment using *Pleurotus florida* to improve the nutritive value and digestibility of rice straw. A total of 40 rice straw samples with and without composting were treated with *P. florida* at different substrate harvesting periods (full ramification, 1st, 2nd, and 3rd flushing) were evaluated by analyzing its chemical composition and *in vitro* digestibility. Their results showed that composted and uncomposted rice straw in the 2nd flush had the highest dry matter (DM) content of 96.07% and 95.56%, respectively. The highest crude protein (CP) content (10.57%) was obtained in composted rice straw in the 3rd flush. The composted rice straw had low organic matter (OM) content (62.63%) but high concentrations of ash (37.37%) and cellulose (46.54%) as compared to un-composted rice straw treated with *P. florida*. These results indicate that the

composted rice straw in the 3rd flush was found to be the best in terms of sufficient degrading of lignin content, minimum loss in cellulose concentrations, enhanced crude protein content, and nutrient digestibility.

Abid *et al.* (2022) conducted *in vitro* studies on crude olive cake, extracted olive cake, and olive leaves using an exogenous fibrolytic enzyme produced from *Trichoderma longibrachiatum* in ruminal nutrition. Treatment was applied by spraying substrates with four doses: 0 (control), 1 (low), 2 (medium), and 4µL/g (high) of dry matter olive mill waste in an air-conditioned room at 26^0 C for 12 h before *in vitro* incubation. Chemical composition of treated olive leaves was found to be CP (10.2, 10.0, 10.5 and 9.7%), cellulose (15.1, 14.9, 14.5 and 14.2%), hemicellulose (14.7, 14.5, 13.1 and 12.7%) and lignin (10.4, 10.3, 10.1 and 10.4%) for 0, 1, 2 and 4µL/g, respectively.

2.3 Extraction and purification of enzymes

Brijwani (2002) investigated micro and macro-scale aspects of solid state fermentation (SSF) for production of cellulolytic enzymes using fungal cultures. In the initial studies surface optimization of SSF of soybeans hulls using mixed culture of *Trichoderma reesei* and *Aspergillus oryzae* was carried out to standardize the process. Optimum temperature, moisture and pH of 30ºC, 70% and 5 were determined following optimization. Using optimized parameters laboratory scale-up in static tray fermenter was performed that resulted in production of complete and balanced cellulolytic enzyme system. The balanced enzyme system had required 1:1 ratio of filter paper and beta-glucosidase units. This complete and balanced enzyme system was shown to be effective in the hydrolysis of wheat straw to sugars. Mild pretreatments with steam, acid and alkali were performed to vary physicochemical characteristics of soybean hulls like bed porosity, crystallinity and volumetric specific surface. Mild nature of pretreatments minimized the compositional changes of substrate. It was explicitly shown that more porous and crystalline steam pretreated soybean hulls significantly improved cellulolytic enzymes production in *T. reesei* culture, with no effect on xylanase. In *A. oryzae* and mixed culture this improvement was not seen. Further studies using standard crystalline substrates and substrates with

varying bed porosity confirmed that effect of physicochemical characteristics was selective with respect to fungal species and cellulolytic activity. A novel deep bed bioreactor was designed and fabricated to address scale-up issues. Bioreactor's unique design of outer wire mesh frame with internal air distribution and a near saturation environment within cabinet resulted in enhanced heat transfer with minimum moisture loss. Enzyme production was faster and levelled within 48 h of operation compared to 96 h required in static tray. A two phase heat and mass transfer model accurately predicted the experimental temperature profile. Simulations also showed that bioreactor operation was more sensitive to changes in cabinet temperature and mass flow rate of distributor air than air temperature.

Godana (2007) evaluated seven fungal strains for their ability to produce xylanase, cellulase and laccase using wheat straw and *Eragrostiscurvula* (outlands grass) as carbon sources in shake flasks. Oulands grass induced high levels of xylanase and cellulase while none of the carbon sources appeared to induce laccase production. *T. aureoviride* (FS2-2) was found to be the best producer of xylanase both on outlands grass and wheat straw (5418 and 4162 nkat / ml, respectively) while Abo 67 (unidentified) was the poor producer (less than 50 nkat / ml) of both xylanase and cellulase. Four fungal strains that showed high enzyme levels in shake flasks were evaluated in SSF for enzyme production using outlands grass as a substrate. Enzyme levels produced in SSF were approximately 6 to 10 fold higher than the levels achieved in SmF depending on the strain used. Maximum xylanase activity achieved in SSF was 14842 nkat / ml produced by *A. fumigatus* followed by *A. terreusvarcarneus* producing 14368 nkat / ml. Supplementing the production media with 0.5% of yeast extract had a positive effect on growth and xylanase production in all strains with *A. fumigatus* resulting in a maximum activity of 17662 nkat / ml after 5 days.

Negi and Banerjee (2009) conducted study with regard to optimization of extraction and purification of glucoamylase produced by *Aspergillus awamori* in solid-state fermentation. Various parameters such as solvent selection, concentration, soaking time, and temperature were tested in a single bioreactor in order to determine optimum extraction conditions of glucoamylase, when

produced simultaneously with protease by *Aspergillus awamari*nakazawa MTCC 6652. Optimum conditions were achieved in a 10% glycerol solution soaked for 2 h at 40°C, followed by concentration of extracted glucoamylase (9,157 U/gds) by acetone precipitation (1:2, v/v), which yielded 51.9% recovery. Ion exchange chromatography and gel filtration showed specific activities of 270.5 and 337.5 U/mg, respectively, while SDS-PAGE and zymogram analysis of glucoamylase indicated the presence of three starch-hydrolyzing isoforms with molecular weights of approximately 109.6, 87.1 and 59.4 kDa, respectively.

Khandeparkar and Bhosle (2012) worked on thermos-alkalophilic *Arthrobacter sp.* that produced extracellular xylanase, when wheat bran, rice husk, rice bran and bagassae were used as carbon source under solid state fermentation (SSF). The xylanase enzyme was isolated by ammonium sulfate (80 %) fractionation, and purified to homogeneity using size exclusion and ion exchange chromatography. The molecular mass of xylanase was ~ 20 kDa. Half-life of enzyme at 70° C and at 60° C was 18 h and 24 h, respectively.

Mrudula and Anitharaj (2011) studied on optimization of process and leaching conditions for improved production of pectinase by *Aspergillus niger* using orange peel as substrate under solid state fermentation (SSF). Among 6 tested substrates, orange peel yielded maximum pectinase. The optimum temperature, pH, incubation time, moisture ratio, inoculum size, carbon source and surfactants, were found to be 50°C, 5, 96 h, 1: 2 (v/w), 2.5 ml, sucrose and Triton-X-1 00, respectively. The strain produced maximum pectinase when a combination of yeast extract and ammonium sulfate was added to the medium. The optimum conditions that influence the extraction of pectinase from the fermented substrate were found to be in presence of sodium acetate buffer, with a volume of 1: 20(w/v), at 50°C with a contact time of 90 min. The partially purified enzyme was characterized and it exhibited maximum activity at a temperature of 50°C and pH 5.

Santos *et al.* (2012) analysed the effects of water content, temperature and time on the kinetic activity of cellulolytic enzymes produced during the solid state fermentation of potato peel, using *Aspergillus niger*. Three main analytical steps –

analysis of variance, regression analysis and plotting of response surface – were performed to obtain an optimum condition for enzymatic activity. The statistical results indicated that the best activity time for enzyme CMCase (carboxymethyl cellulase) is 82.88 h, with water content of 51.48% and temperature of 29.46 ^{o}C; for FPase (filter paperase), the best activity time is 80.62 h, water content of 50.19% and temperature at 30.00^{o}C; for xylanase, time is 81.92 h, water content is 50.72% and temperature is 28.85^{o}C. Pareto charts have shown that all variables were significant in enzymatic activity for CMCase and xylanase. On the other hand, FPase showed that time and temperature have significant effect for this response variable.

Udenwobele *et al.* (2014) studied on pectinase production from a culture of *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus flavus*. Pectinase synthesis was achieved using mango (*Mangifera indica*) pectin extract as an inducer during pectinolytic fungi isolation while submerged fermentation process was carried out using ground mango peels as the sole carbon source. Substrate fermentation was evaluated within seven days by monitoring the pectinase activity every 24 h. The highest pectinase secretion was obtained from *A. niger* and *A. fumigatus* after 92 h (day 4) of incubation, while in *A. flavus,* it was after 120 h (day 5). Crude enzyme extracts from the three organisms were partially purified by a combination of ammonium sulphate precipitation and dialysis with an approximately two-fold purification of the pectinase and a yield of 5.4, 7.66 and 5.99% for *A. niger, A. fumigatus* and *A. flavus*, respectively after dialysis. The specific activities of 1.62, 1.79 and 1.86 U/mg for *A. niger, A. fumigatus* and *A. flavus* enzymes were calculated, respectively. The results suggested that mango peels can be used for value added synthesis of pectinase with numerous biotechnological applications.

Joshi *et al.* (2015) studied the extraction of enzymes from potato peels substrate using *Bacillus Subtilis*. The work was done to study the growth habit of microbes on standard media, standardize the conditions for growth of microbes using potato peel and measure the efficacy of filtrate as enzyme source. Experimental details were like Design-CRD, 3 replication and 8 treatments.

Bacterial strains were used like B1 = *B. subtilis*, B2= No bacteria, Incubation temperature T1 = 37°C, T2 = Room Temperature (R.T.) and Incubation Hours H1 = 24, H2 = 48. Bacterial strain *Bacillus subtilis* consume maximum amount of starch (96.27 mg/g) as compare to other treatments. Amylase and protease enzymes activity also found the highest in treatment. All the results were within accepted criteria.

Wang *et al.* (2018) performed experiment on extraction of enzyme used to extract the polysaccharides from pumpkin seeds (PSP) and the extraction parameters were optimized by response surface methodology (RSM). Under the optimum experimental parameters: Extraction temperature of 60°C, extraction time of 43 min, enzyme concentration of 2.5%, and pH of 6.0, the yield of PSP was 3.22 ± 0.04%, which was in close agreement with the predicted value (3.24%). After further purification on anion exchange column and gelfiltration column, a novel purified polysaccharide (PSPE) with molecular weight of 16,700 g/mol was obtained. PSPE was mainly composed of mannose, galactose and glucose in the molar ratio of 1.00:3.84:1.62.

Sulyman *et al.* (2020) made the isolation, purification and characterization study of cellulase produced by *Aspergillus niger* cultured on *Arachis hypogaea* shells. The crude cellulase enzyme was produced by *A. niger* through submerged fermentation process using *A. hypogaea* shells as a carbon source. The crude cellulase was purified through ammonium sulphate precipitation, dialysis and gel-filtration chromatography. The molecular weight was estimated using sodium dodecyl sulphate polyacrylamide gel electrophoresis. The effects of pH and temperature on the activity of the purified cellulase were investigated. The study revealed that the optimal production of crude cellulase was achieved at incubation period of 120 h, pH 4, temperature 40°C, and inoculum size of 13 x 10^5 CFU/ml. Cellulase was purified to 68.12-fold with a yield and specific activity of 3.87% and 484.3 U/mg, respectively. The V_{max} for the cellulase was 9.26 U/ml while the K_m was 0.23 mg/ml. The optimum pH and temperature for the cellulase activity were 4 and 40^0C, respectively. Their study has shown that *Arachis hypogaea*

shells can be used as a carbon source by *Arachis niger* for the production of cellulase.

2.4 Quantitative analysis of enzymes

Delabona *et al.* (2012) produced cellulolytic enzymes includes both microorganism selection and improved fermentation process conditions. Work includes the isolation, screening and selection of biomass-degrading fungi species from the Amazon forest and analyzes the enzymatic complex produced by a selected strain of *Aspergillus fumigatus* cultivated using different agro-industrial residues like wheat bran, sugarcane bagasse, soybean bran, and orange peel as substrate in solid state fermentation (SSF). Enzyme activities up 160.1 IU/g for CMCase (endoglucanase), 5.0 FPU/g for FPAse, 105.82 IU/g for β-glucosidase and 1055.62 IU/g for xylanase were obtained during 120 h of cultivation.

Salihu *et al.* (2015) studied on different agricultural residues for their ability to support cellulolytic enzyme production by *Aspergillus niger*. A total of eleven agricultural residues including finger millet hulls, sorghum hulls, soybean hulls, groundnut husk, banana peels, corn stalk, cassava peels, sugarcane bagasse, saw dust, rice straw and sheanut cake were subjected to three pre-treatments (acid, alkali and oxidative) methods. All the residues supported the growth and production of cellulases by *A. niger* after 96 h of incubation. Maximum cellulase production was found in alkali-treated soybean hulls with CMCase, FPase and β-glucosidase yields of 9.91 ± 0.04, 6.20 ± 0.13 and 5.69 ± 0.29 U/g, respectively.

Kholif *et al.* (2017)[a] determined the effect of anaerobic ensiling of raw agricultural waste with a fibrolytic enzyme cocktail as a cleaner and sustainable biological product for animal feed. Ten samples of one kilogram of wheat straw, corn stalks and sugarcane bagasse were ensiled with enzyme cocktail at 0, 1 or 3 ml/kg dry matter of feed. Fodder ensiling with enzyme cocktail of activity 7.1 unit/g cellulases, 2.3 unit/g xylanases, 61.5 unit/g α-amylase and 29.2 unit/g protease and observed the fibrolytic effects by evaluation of feed nutrient content and in vitro gas and methane production.

Ijoma *et al.* (2018) investigated biological inducing effect on production of lignolytic enzymes by fungal co-cultures. The study examined the effect of antagonistic invasion as a scheme to hasten the production of secondary metabolites, thus increasing lignolytic enzyme activity, while using substrates like corn cob, wheat straw and sugarcane bagasse. Their results indicated an increment in enzyme production upon antagonistic dual cultivation of fungi, thereby proving the initial hypothesis. Analysis of the average enzyme activity values revealed that *Trichoderma sp.* partnered in dual cultures, producing manganese peroxidase at a rate of about 1.46 U/mL as opposed to 0.06 U/mL in a monoculture. Furthermore, compared to monocultures of 0.05 U/mL, dual culture laccase concentrations were roughly 0.09 U/mL.

Astolfi *et al.* (2019) evaluated the production of cellulolytic enzymes from different agricultural residues for application in biofuel production. The crude enzyme extract produced was characterized and applied for saccharification of some agricultural residues. Maximum cellulolytic activities were obtained using soybean hulls. The generation of FPase peaked after three days of fermentation with soybean hulls (6.71 U/g), persisted for nine days (6.62 U/g), and then started to fall by around 30% until day fifteen. However, a maximum output of 4.06 U/g was found after 6 days for the mixed fermentation of soybean hulls and powder dried yerba mate (1:1), a result that was 40% lower than when soybean hulls were used.The maximum activity of CMCase was found in 6 days (20.77±0.02 U/g) for fermentation with mixed substrate. Activity of xylanase production high for soybean hulls on the third day; however, the maximum value of 1,130.70 U/g was obtained in 6 days of fermentation. Then the activity decreased to about 40% (648.38 U/g) on the fifteenth day. The fermentation with the mixed substrate observed maximum production of 581.72 U/g at 3 days.

2.5 Degradation degree testing for enzyme

Wierzba and Nabrdalik (2007) conducted study on biodegradation of bottom sediments of Turawa Lake. In the cellulolytic bacteria strains were employed and the biodegradation assumed 19% reduction of the total cellulose content. At the beginning cellulolitic bacteria, originating from the bottom sediments, were

isolated and selected. Next step involved evaluation of the cellulolitic activity of bacteria strains and selection of the three most vigorous, to be used in the process of bottom sediments biodegradation. The amount of decomposed cellulose was assessed with the use of anthrone method and changes in bacteria enumeration were determined with the index method. The most efficient group, regarding biodegradation of cellulose, was the mixture of the bacteria species Cytophaga and Cellulomonas. The reduction obtained was equal to 66%, and the bacteria enumeration increased 300-fold, to finally reach the level of the Most Probable Number equal to 105 NPL per 1g of the bottom sediment.

Yoshida *et. al.* (2008) investigated the effects of cellulose crystallinity, hemicellulose, and lignin on the enzymatic hydrolysis of *Miscanthuss inensis* to monosaccharides. A air dried biomass was ground by ball-milling, and the powder was separated into four fractions by passage through a series of sieves with mesh sizes 250–355μm, 150–250 μm, 63–150μm, and < 63 μm. Each fraction was hydrolyzed with commercially available cellulase and β-glucosidase. The yield of monosaccharides increased as the crystallinity of the substrate decreased. The addition of xylanase increased the yield of both pentoses and glucose. Delignification by the sodium chlorite method improved the initial rate of hydrolysis by cellulolytic enzymes significantly, resulting in a higher yield of monosaccharides as compared with that for untreated samples. When delignified *M. sinensis* was hydrolyzed with cellulase, β-glucosidase, and xylanase, hemicellulose was hydrolyzed completely into monosaccharides, and the conversion rate of glucan to glucose was 90.6%.

Abidi *et. al.* (2010) conducted study on two cotton cultivars TX19 and TX55 (*Gossypiumhirsutum L. cv*.) planted in the greenhouse and fibers were harvested at different stages of development. The percentage of sugars present on the fibers was determined by HPLC and the cellulose content was determined using the anthrone method. The percentage of sugars (sucrose, glucose, fructose, and galacturonic acid) showed statistically significant changes during fiber development. The decrease in the percentages of sugars as the secondary cell wall develops was associated with an increase in the cellulose content.

Choi *et al.* (2010) studied the enzymatic degradation on algal biomass, *Chlamydomonas reinhardtii* UTEX 90 that was converted into a suitable fermentable feedstock by two commercial hydrolytic enzymes. Their results showed that all starch was released and converted into glucose without steps for the cell wall disruption. As a result, approximately 235 mg of ethanol was produced from 1.0 g of algal biomass by a separate hydrolysis and fermentation (SHF) method. For tracking total carbohydrates resulting due to enzymatic pre-treatment, the authors employed the anthrone method showing 59.7±0.5 % total carbohydrate composition. In the study, they could correlate the increased bioavailability of reducing sugars with increase in unfolding of complex lignocellulosic structures due to enzyme action.

2.6 Thermostability of enzymes

Delabona *et al.* (2012) produced cellulolytic enzymes with both microorganism selection and improved fermentation process conditions. They analyzed the enzymatic complex produced by a selected strain of *Aspergillus fumigatus* cultivated using different agro-industrial residues like wheat bran, sugarcane bagasse, soybean bran, and orange peel as substrate in solid state fermentation (SSF). Studies of this extract showed that the CMCase was most active at either 65°C or pH 3-3.5, indicating that microorganism produces a thermophilic and acid endoglucanase.

Astolfi *et al.* (2019) evaluated the production of cellulolytic enzymes from different agricultural residuesfor application in biofuel production. The crude enzyme extract produced was characterized and applied for saccharification of some agricultural residues. Maximum cellulolytic activities were obtained using soybean hulls as a substrate, *Trichoderma reesi* was inoculated for solid substrate fermentation. All enzymatic activities were highly stable at 40°C at a pH range of 4.5–5.5. For stability at low temperatures, the enzyme extract was stored at freezing temperature and cooling for about 290 days without major loss of activity.

2.7 *In vitro* Nutrients Digestibility

Bunting (1982) studied *in vitro* dry matter digestibility of untreated sorghum stover, ozonated sorghum stover, and a 50/50 mixture of treated and untreated sorghum stover. *In vitro* dry matter digestibility (%) of ozone treated stover (39.63±0.71, 38.24±0.54) and the 50/50 treatment (50.33±0.59, 49.48±0.38) showed non-significant difference ($p<0.05$) at 24 and 48 hr of incubation, however, both treatments were superior ($p<0.05$) than untreated stover (37.08±1.26, 44.62±0.75) at 24 and 48 hr of incubation. IVADFD (%) of ozone treated sorghum stover had significant (42.02+0.45) differences ($p<0.05$) as compared to 50/50 mixture of treated (40.37±0.85) and untreated sorghum stover (36.18±1.20) at 24 hr of incubation, however, non-significant difference existed in ozone treated stover (43.81±1.74,) and the 50/50 treatment (44.85±0.81) than untreated sorghum stover (36.76+1.60) at 48 hr of incubation.

Okamoto *et al*. (2001) studied the effect of ozone treatment on the feed value of cotton stalk, wheat straw and rice straw. The ozonizing periods were 30 min. 60 min and, 120 min. *In vitro* dry matter digestibility value of cotton stalk of ozonation time were found as 36.7, 41.2 and 45.0% as compared to untreated cotton stalk (24.3%).

Hamza *et al.* (2005) carried out an experiment to study the effect of incubated cotton stalks and rice straw with white rot fungi growth (*Pleurotus ostreatus*) on the digestibility (*in vitro* and *in vivo*) and feeding value of cotton stalks and rice straw. Results showed that the *in vitro* dry matter and organic matter digestibility of treated rice straw (33.87 and 43.22%) and cotton stalk (36.06 and 44.37%) were significantly increased ($p<0.05$) as compared to untreated rice straw (26.08 and 31.39%) and cotton stalks (29.27 and 32.54%), respectively. They concluded that biological treatment could be used successfully to enrich the poor quality roughages and improved digestibility of treated materials.

Yu *et al.* (2005) studied on *in vitro* dry matter digestibility on oat hulls for improving the nutritional value for ruminant animals with pretreatment of a

multienzyme cocktail. In Exp. 1, the best multienzyme cocktail (ferulic acid esterase, xylanase, cellulase, endo-glucanase [I, II], and β-glucanase) was developed using an orthogonal experimental design. In Exp. 2, *in vitro* biodegradation studies with a 3 × 2 × 4 factorial arrangement of treatments were used to evaluate the responses of three feedstuffs, oat hulls or standard references (wheat straw and alfalfa hay), two particle sizes (1 mm and 250 µm), and four *in vitro* incubation treatments with the best multienzyme cocktail developed in Exp. 1. They observed that, addition of the multienzyme cocktail to the forages improved *in vitro* dry matter disappearance with respect to feedstuff, the order of response ($p < 0.05$) of oat hulls (27.7, 34.8%), wheat straw (29.9, 31.7%) and more in alfalfa hay (60.1, 62.6%) in 1 mm and 250 µm particle size in combination incubation treatments, respectively. They concluded that the addition of the multienzyme cocktail to poorly digestible feeds before feeding enhanced degradation of dry matter.

Ravhuhali *et al.* (2010) determined the chemical composition and *in vitro* enzymatic digestibility of four cowpea cultivars and buffalo grass hay. The four cowpea cultivars used were *Pan 311*, *Red caloona*, *Black eye*, *Agripes* and buffalo grass hay. Analysis of variance indicated that *in vitro* dry matter digestibility of four cowpea cultivars had higher (74, 75, 70 and 71%) than buffalo grass hay (55%). Percentages of IVNDFD (39, 40, 37 and 33) and IVADFD (25, 25, 26 and 22) of four cow pea cultivators significantly ($p<0.05$) higher than buffalo hay grass (26 and 19), respectively.

Ganai *et al.* (2011) evaluated the effects of exogenous fibrolytic enzymes supplemented to bajra straw @ 0, 1, 2, 3 and 4 g/kg substrate dry matter on degradability of DM and NDF as well as total gas production (TGP) in T_0, T_1, T_2, T_3 and T_4 group using goat rumen liquor in first experiment. There was significant ($p<0.01$) improvement in nutrient degradability of DM and IVNDFD in bajra straw in T_1 (44.63, 31.07%), T_2 (50.35, 35.29%), T_3 (48.55, 34.50%) and T_4 (47.84, 33.18%) as compared to control (41.51, 29.62%) on 48 hr incubation. In second experiment, *in vitro* trial was performed to evaluate the effects of exogenous fibrolytic enzymes (2 g/kg DM) on rumen fermentation characteristics

in complete feed containing bajra straw 60 parts and concentrate mixture 40 parts. They observed significant ($p<0.01$) improvement in IVDMD and IVNDFD in enzyme supplemented (60.29, 50.78%) compared to control (51.78%).

Salem *et al.* (2011) studied the effect of exogenous enzymes of ZADO® (i.e., ENZ) and on nutrients digestibility and growth performance in six crossbred sheep (32.00±0.603 kg body weight (BW)) and 6 Baladi goats (18.00±0.703 kg BW) in 2 × 2 factorial design. Animals were fed on wheat straw *ad libitum* and restricted amount of commercial concentrate with (+ENZ) or without (-ENZ) 10 g/animal/day of ZADO® to cover 120% of their maintenance requirements. The *in vitro* dry matter digestibility (%) of wheat straw in absence and presence of enzymes found 68.77 and 81.57 % for 48 hr incubation.

Thakur and Shelke (2011) studied the effect of different periods of storage and temperatures of total mixed rations (TMR) containing exogenous fibrolytic enzymes (EFE) on enzyme activity and *in vitro* digestibility. Cellulase (4026 µM glucose/g/h) and xylanase (7825 µM xylose/g/min) were mixed in equal proportion (w/w) and added at 1.5 and 3.0 g/kg DM of TMR to the concentrate portion of TMR. The concentrates were subjected to heating at 40, 50, 60 and 80°C in a hot air oven for 2, 5 and 10 minutes in first experiment and stored in airtight polythene bags at room temperature (30-34^{0}C) for 0, 7, 15, 30, 45 and 60 days in another experiment. Thereafter, concentrates containing EFE were mixed with wheat straw and green fodder (sorghum) in 25:60:15 (TMR I), 40:45:15 (TMR II) and 60:25:15 (TMR III) ratios on DM basis and IVDMD, IVNDFD and IVADFD were estimated at 48 hours post incubation. They did not observe adverse effect of heating on cellulase and xylanase activities, irrespective of temperature and heating time as well as storage up to 60 days. Similarly, there was no effect of heating concentrate containing 1.5 g and 3.0 g EFE/kg DM to 40, 50, 60 and 80^o C for 2, 5 and 10 minutes or storing up to 60 days on percent IVDMD, IVNDFD and IVADFD of the three TMRs containing these concentrates. They concluded that there was no adverse effect of storage (up to 60 days) and heating (up to 80^{0}C) of total mixed rations containing fibrolytic enzymes on cellulase and xylanase activities and *in vitro* fiber digestibility.

Bhaskar *et al.* (2012) studied the effect of fibrolytic enzyme cocktail comprising of cellulase, xylanase and β-D glucanase for sorghum stover based on *in vitro* DM digestibility (IVDMD) using rumen liquor from cattle maintained on sorghum stover. At first, cellulase and xylanase were added individually to ground sorghum stover at an increasing dose rates (100, 200, 400, 800, 1600, 3200, 6400, 12800, 25600, 32000, 38400, and 44800 IU/g) subjected to higher IVDMD. The doses attributed to higher IVDMD ($p<0.01$) for cellulase (32000, 38400 and 44800 IU/g) and xylanase (12800 and 25600 IU/g) were selected for formulating cellulose-xylanase combinations and the enzyme combinations (cellulase-xylanase, IU/g) with higher IVDMD ($p<0.01$) for cellulase (32000, 38400 and 44800 IU/g) and xylanase (12800 and 25600 IU/g) were selected for formulating cellulasex-ylanase combinations and the enzyme combinations (cellulase-xylanase, IU/g) with higher IVDMD ($p<0.01$) selected were 32000 and 25600, 38400 and 12800, 44800 and 12800 and 44800 and 25600 IU /g. To these combinations β-D glucanase was added at (0, 100, 200, and 400 IU/g) and a synergistic effect of β-D glucanase was observed. Based on *in vitro* studies their study indicated that exogenous fibrolytic enzyme cocktails (cellulase- xylanase-β-D glucanase, IU/g) 32000 – 25600 - 400 or 38400 - 12800 - 100 was optimum for enhancing nutrient utilization from sorghum stover for large ruminants.

Wang *et al.* (2012) studied the effects of exogenous fibrolytic enzymes (EFE a mixture of two preparations from *Trichoderma spp.*, with predominant xylanase and β-glucanase activities, respectively) on colonization and digestion of ground barley straw and alfalfa hay by *Fibrobacter succinogenes* S85 and *Ruminococcus flavefaciens* FD1 *in vitro.* DM disappearance (DMD), was higher ($p<0.01$) with ammoniated (5% w/w; AS) than with native (S) ground barley straw. Application of EFE to the straws increased ($p<0.01$) ^{15}N-PAMN at 4 h only, but EFE on AS increased ($p<0.01$) DMD from both S and AS at 4 and 12 h, but reduced ($p<0.01$) ^{15}N-PAMN in the early stage (4 h) of the incubation, as compared to non-pre-hydrolysed samples.

Diaz *et al.* (2013) assessed the effects of four doses of three commercial fibrolytic enzymes on ruminal fermentation of rice straw, maize stover and

*Pennisetum purpureum*clon Cuba CT115 hay in batch cultures of ruminal micro-organisms from sheep. One enzyme was produced by *Penicillium funiculosum* (PEN) and two were from *Trichoderma longibrachiatum* (TL_1 and TL_2). Each liquid enzyme was diluted 200 (D_1), 100 (D_2), 50 (D_3) and 10 (D_4) - fold and applied to each substrate in quadruplicate over time and incubated for 120 hr in rumen fluid. In 9 h incubations, PEN at D_4, TL_1 at all tested doses, and TL_2 at D_2, D_3 and D_4 increased ($p<0.05$) dry matter degradability for all substrates.

Torres *et al.* (2013) evaluated an enzymatic extract from *Cellulomonas flavigena*at 0, 2.5, 7.5, 12.5 ml/kg DM of total mixed ration (TMR) on the *in vitro* degradation of DM, NDF and ADF for incubation of 6, 12, 24, 48 and 72 hr and *in vivo* at 0, 5.0 and 7.5 ml of extract per kg DM of TMR to determine the digestibility and productive performance of lambs fed a TMR made up of 60% forage. Twenty-four Pelibuey-Kathadin lambs were used in the trial. The *in vitro* dry matter digestibility (%) showed lower ($p<0.05$) in enzymatic extract dose 2.5, 7.5, 12.5 ml/kg DM (68.3, 68.3, 68.5%) than 0 ml /kg DM (71.1%) whereas IVNDFD lower in 2.5, 7.5 ml/kg DM (78.7, 77.8%) as compared to 0, 12.5 ml/kg DM (80.7, 80.1%), while IVADFD significantly higher ($p<0.05$) in 7.5, 12.5 ml/kg DM (67.3, 68.6%) followed by 0, 2.5 ml/kg DM (64.0, 62.7%), respectively at 48 hr of incubation.

Rajamma *et al.* (2015) studied the effect of inclusion of exogenous fibrolytic enzymes (EFEs) in feeds identified as a promising alternative to improve energy availability for ruminants. Maize stover was used as basal roughage, which was oven dried at70ºC and finely ground in Willey mill using 1 mm sieve. Two iso-nitrogenous total mixed rations (TMR) with 11% crude protein (CP) contentwere prepared containing 60% maize stover (TMR-1) and 70% maize stover (TMR-2) as core ingredient. The exogenous fibrozyme (Fermentation extracts of *Aspergillus niger* and *Trichoderma viride*, containing cellulases and hemicellulases @ 100 IU as xylanase/g) was combined with TMR-1 and TMR-2 @ 2.5g/kg TMR (on DM basis). Four dietary treatments (T_1, T_2, T_3, T_4), containing two different types of roughage to concentrate ratio (R:C), supplemented with and without EFE

(control), viz., T_1 (60R:40C), T_2 (T1 + EFE), T_3 (70R:30C), and T_4 (T_3 + EFE) were subjected to trial, using rumen liquor, collected from two rumen fistulated buffalo bulls fed on these diets. The *in vitro* digestibility of dry matter (IVDM), crude protein (IVCP), neutral detergent fiber (IVNDF), and acid detergent fiber (IVADF) were evaluated. Their results indicated that type of ration (TMR-1 and TMR-2) had no significant ($p>0.05$) effect on IVDMD, EFE supplementation significantly ($p<0.05$) improved IVDMD. There was significant ($p < 0.01$) interaction between the R:C ratio in the ration and EFE supplementation. The digestibility (%) of IVDM (62.18), IVNDFD (60.04) and IVADFD (52.03) of T_2 treatment (60R:40C) were significantly ($p<0.01$) higher than the digestibility (%) of IVDM (59.66), IVNDFD (58.13) and IVADFD (49.04) of T_4 treatment (70R:30C). It was concluded that supplementation of diet with exogenous fibrolytic enzymes significantly increased nutrient digestibility of the ration.

Zhao *et al.* (2015) observed the effect of commercial exogenous fibrolytic enzymes on *in vitro* rumen fermentation and methane production with corn stover as substrate. Two in vitro experiments were conducted using reading pressure technique system. In Exp.1, synergetic effect of cellulase (CEL) (0, 10, 20 and 30 U/g DM) and xylanase (XYL) (0, 20, 40 and 60 U/g DM) were evaluated on corn stover by a 4 × 4 factorial design. In Exp.2, the effect of two chosen enzymes were evaluated on 3 corn stover based mixed diets by a 3 × 3 factorial design. In Exp.1, *in vitro* dry matter digestibility (IVDMD) and IVNDFD of a single 30U/g of DM of CEL (48.5 and 36.9%) and combination of 10 U/g of DM of CEL with 60 U/g of DM of XYL (49.3 and 37.7%) were screened out as a higher. In their study, the fibrolytic enzyme supplement potentially improved fiber digestion of corn stover in this *in vitro* experiment.

Reddy *et al.* (2016) observed the effect of supplementation of exogenous fibrolytic enzyme (EFE) or live yeast culture or both in total mixed rations on digestibility of nutrients. The dietary treatments included a groundnut haulms based total mixed ration (TMR) with R: C ratio of 70: 30 (T_1), T_1 supplemented with EFE @ 15 g/animal/d (T_2), T_1 supplemented with live yeast culture @ 10

g/animal/d (T_3) and T_1 supplemented with EFE @ 15 g/animal/d and live yeast culture @ 10 g/animal/d (T_4). Results indicated that the *in vitro* digestibility (%) of DM, NDF and ADF was lower ($p<0.05$) in T_1 (50.97, 49.45 and 46.4%) as compared to T_2 (55.69, 54.41 and 51.94%), T_3 (56.63, 55.28 and 53.56%), and T_4 (57.02, 57.28 and 55.00%). Further, the *in vitro* digestibility (%) of DM, NDF and ADF increased linearly from T_2 to T_4, but the differences were not significant ($p>0.05$). They concluded that supplementation of either EFE or yeast culture or both increased the *in vitro* digestibility of nutrients.

Santoso *et al.* (2016) evaluated the *in vitro* nutrient digestibility and fermentation characteristics of king grass combined with a concentrate that contained mixed microbes. *Lactobacillus plantarum, Saccharomyces cerevisiae* and two strains of cellulolytic bacteria (i.e., *Acinetobacter baumannii* and *Pseudomonas aeruginosa*) were added to the concentrate. Four concentrates were made: A, concentrate without microbe; B, concentrate containing *L. plantarum* and *S. cerevisiae*; C, concentrate containing *L. plantarum*, *S. cerevisiae* and *P. aeruginosa* and D, concentrate containing *L. plantarum*, *S. cerevisiae* and *A. baumannii*. Bacteria and yeast were added to the concentrate at 10^6-10^7cfu/g. *In vitro* nutrient digestibility assays were conducted using 250 mg substrate composed of king grass and concentrate (70: 30, DM). Results revealed that *in vitro* dry matter digestibility (IVDMD) was higher ($p<0.01$) for the grass substrate concentrate without microbes (44.1%) and concentrate containing *L. plantarum* and *S. cerevisiae* with grass substrate (44.5%) compared with control feed (39.2%). NDF digestibility was greater ($p<0.01$) for the grass substrate combined with concentrate that contained mixed microbes (43.8, 43.4 and 45.1%) compared with concentrate without microbes (35.4%). They concluded that addition of mixed microbes to the concentrate improved the digestibility of nutrients *in vitro.*

Hashim *et al.* (2017) studied chemical composition of both Bt and non-Bt cotton crop residues (CCR) and *in vitro* digestibility of each type of CCR was also determined. Week zero represents the period before introduction of animal to graze on Bt-CCR. From each square meter of 14 square meter cotton residues were cut at 10 cm above the ground level. The components of the crop residues in

each square meter were separated in to leaves, bolls and tender branches and then weighed freshly and again after drying in an oven at 105^0C for 24hours. The results revealed significant differences in *in vitro* dry matter digestibility in Bt CCR. The *in vitro* digestibility showed lower levels in Bt CCR (46.00, 41.33 and 38.67%) than in non-Bt CCR (60.00, 56.00 and 54.00%) in first, second and third week study.

Lunagariya *et al.* (2017) studied the effect of levels of exogenous fibrolytic enzymes (EFE) on *in vitro* digestibility of dry matter (DM) and organic matter (OM), total gas production (TGP), metabolizable energy (ME) content, and microbial biomass production (MBP). The total mixed ration (TMR) was prepared using 30% each of sorghum hay and groundnut straw and 40% compound concentrate mixture to meet nutritional requirement of cow. The EFE was incorporated at 0, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, and 400 mg/kg TMR. The significantly ($p<0.05$) higher and optimum *in vitro* digestibility of DM (63.03%) was observed at supplementation of 240 mg EFE/kg TMR.

Santoso *et al.* (2017) evaluated the nutritive value, *in vitro* fermentation characteristics and nutrient digestibility of agro-industrial byproducts-based complete feed block enriched with mixed microbes. The complete feed blocks were mainly composed of agricultural and food industry byproducts such as rice straw, palm oil frond, tofu waste, cassava waste, sago starch and molasses. Four treatments were A, complete feed block without microbe; B, complete feed block containing *Lactobacillus plantarum*, *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa*; C, complete feed block containing *L. plantarum, S. cerevisiae* and *Acinetobacter baumannii*; D, complete feed block containing *L. plantarum*, *S. cerevisiae, P. aeruginosa* and *A. baumannii*. All complete feed blocks were formulated to be isonitrogenous. Crude protein content was similar (11.6%) for 4 feed blocks. *In vitro* dry matter digestibility (IVDMD) and IVNDFD were found that feed blocks containing mixed microbes B (54.4, 35.5%), C (55.6, 35.3%) and higher in D (58.2, 38.3%) of complete feed block containing combination of *L. plantarum, S. cerevisiae, P. aeruginosa* and *A. baumannii* as compared to

treatment A (51.7, 31.4%) complete feed block without microbe, respectively.

Sheikh *et al.* (2017) evaluated the effects of probiotic mix and fibrolytic enzyme mixture on *in-vitro* fermentation and digestibility using whole rumen flora from sheep. The feed additives viz. probiotic mix and fibrolytic enzyme mixture were incorporated @ 3g, 6g, 9g, 12g, 15g/kg dry matter of complete feed for adult sheep. The rations were subjected to *in vitro* analysis to evaluate DM digestibility for determination of optimum level of incorporation of feed additives in the complete ration. Significant improvement in degradability of DM (IVDMD) and NDF (IVNDFD) of paddy straw was observed due to probiotics mix (40.00, 38.40 and 31.20, 29.30%) and fibrolytic enzyme mixture (37.68, 40.05% and 28.45, 32.10%) supplementation with maximum values at 3 g and 9 g/kg DM.

Santoso *et al.* (2018) evaluated *in vitro* nutrient digestibility, fermentation characteristics and methane production of agro-industrial byproducts-based complete feed block treated with mixed microbes. Five treatments were A: complete feed block without microbe (control); B: complete feed block containing 1.5% *L. plantarum*,1.5% *S. cerevisiae* and 1% *P. aeruginosa*; C: complete feed block containing 1.5% *L. plantarum*,1.5% *S. cerevisiae*, 2% *P. aeruginosa* and 2% *A. baumanni*; D: complete feed block containing 1.5% *L. plantarum*, 1.5% *S. cerevisiae*, 1% *P. aeruginosa* and 1% *A. baumanni*; E: complete feed block containing 1.5% *L. plantarum*, 1.5% *S. cerevisiae*, 2% *P. aeruginosa* and 2% *A. baumanni*. Results showed that *in vitro* dry matter digestibility % (IVDMD) of feed blocks containing mixed microbes were found 55.6, 56.6, 55.6 and 56.6% in B, C, D and E, respectively have non-significance difference ($p<0.01$) with complete feed block without microbe (53.8%) and IVNDFD (%) found to be higher in 2% of *P. aeruginosa* and *A. baumanni* (C and E) in complete feed block as compared to others.

Muruz *et al.* (2019) determined the effects of a mixed bacterial inoculant possessing ferulic acid esterase (FAE) activity and exogenous fibrolytic enzyme (EFEs) products on the chemical composition, conservation characteristics and digestibility of corn stover. The corn stover treated with deionized water (control), EFEs (10 U cellulase + 60 U xylanase units/g of substrate dry matter) or EFEs +

FAE inoculant (FAEI) (1.3x105 cfu/g fresh forage). The treated corn stover was then incubated in laboratory mini-silos for 30 days. The *in vitro* true dry matter digestibility (IVTDMD) and IVTNDFD of the corn stover treated with EFEs (63.70 and 43.70%) and EFEs + FAEI (64.32 and 44.62%) were higher than for the control (59.87 and 38.54%) ($p<0.01$), respectively but there was no significant difference between the EFEs and EFEs + FAEI treatments. EFEs played a major role in enhancing the digestibility of corn stover, alone or in combination with FAEI.

Balakesava Reddy *et al*. (2020) conducted an experiment to evaluate paddy straw treated with maize spent liquor (MSL) at 10, 20 and 30% and subjected to different days (1, 2, 3, 4 and 5) of incubation for *in vitro* digestibility of nutrients. Data revealed that *in vitro* digestibility (%) of dry matter of paddy straw treated with MSL at 10 and 20% increased linearly ($p>0.05$) from 1 to 5 days of incubation (48.31$\pm$0.61 and 49.39$\pm$0.36), respectively, but decreased at 30% level of inclusion (49.18$\pm$0.40). Higher *in vitro* digestibility of dry matter (50.10%), was observed in paddy straw treated with 20% MSL and incubated for one day compared to other treatments. The study concluded with better *in vitro* digestibility of dry matter in paddy straw treated with MSL at 20% level and incubated for one day.

Amido *et al.* (2021) determined the potentiality of the composting process and biological pretreatment of using *Pleurotus florida* to improve the nutritive value and enhance the digestibility of rice straw. A total of 40 rice straw samples with and without composting treated with *P. florida* at different substrate harvesting periods were evaluated by analyzing its chemical composition and *in vitro* digestibility. Results showed that the *in vitro* dry matter digestibility (IVDMD), IVNDFD and IVADFD of composted rice straw was higher (62.54, 52.89, 50.10%) than in uncomposted rice straw (61.54, 52.52, 47.63%) treated with *P. florida* ($p<0.05$), respectively.

Abid *et al.* (2022) conducted *in vitro* study on crude olive cake, extracted olive cake, and olive leaves using an exogenous fibrolytic enzyme produced from *Trichoderma longibrachiatum* in ruminal nutrition. Treatment was applied by

spraying substrates with four doses: 0 (control), 1 (low), 2 (medium), and 4µL/g (high) of dry matter olive mill waste in an air-conditioned room at 26° C for 12 h before *in vitro* incubation. Upon *in vitro* incubation of olive leaves, the medium dose increased the dry matter degradability by 13% (66.1%) compared with the control (58.3%).

2.8 Effect of Complete Feed on Body Weight and Average Daily Gain

Yadav and Deshmukh (2001) conducted an experiment to evaluated the use of spent straw in complete ration of sheep. In complete feed they maintained wheat straw (CR-1) and spent straw (CR-2) at 60% level. For CR-1 and CR-2, the DCP and TDN contents were 5.35, 56.10 and 7.09, 41.68 percent, respectively. At the end of the trial, both meals were shown to be capable of maintaining experimental sheep with an average daily gain of 50-60 g.

Raut *et al*. (2002) evaluated arhar (*Cajanus cajan*) straw based complete feed pellets in local non-descript male goats. The goats were fed with pelleted complete diet containing 60% *Cajanus cajan* straw and 40% concentrate mixture (jowar 30, cotton seed cake 27, arhar chunni 30, groundnut cake 10, mineral mixture 2 and common salt 1 percent). The results of the experiments demonstrated that a full meal based on pigeon pea straw could keep experimental goats gaining an average weight of 75g per day.

Kirubanath *et al.* (2003) examined the effect of processing cotton straw based complete diet with expander-extruder on performance of crossbred calves. For this experiment, eighteen crossbred calves (6-9 months, 73.48 6.52 kg) were randomly assigned to two full diets and a conventional diet (6 in each group) for 180 days in a growth trial. The complete diets were prepared with 40% cotton straw, one processed in mash form and the other pelletized using an expander-extruder (EEP). These two full meals were compared to a traditional feeding method in which cotton straw and concentrate mixture were fed separately in a 60:40 ratios. The total gain in weight under standard, mash and EEP conditions were 83.68, 112.87 and 146.74 kg, respectively. In compared to conventional

meals, the ADG was considerably ($p<0.01$) higher in calves fed EEP complete feeds (815.4 g) followed by mash (627.0 g) and conventional (464.9 g) system.

Reddy and Reddy (2003) investigated the effect of a cotton stalks-based complete diet on growth characteristics in sheep and goats in field conditions, a comparison trial was undertaken on 6 Nellore ram lambs and 6 local male youngsters (4–5 months old). For 180 days, they were fed an expander extruder pressed pelleted complete feed prepared with cotton stalks as the only source of roughage (40%) and concentrates (60%). The ADG in lambs and goats was 102.8 and 81.1 g, respectively, which was considerably greater ($p\leq0.05$) in lambs than goats.

Gad *et al.* (2011) studied the effect of replacing berseem hay (BH) with corn stalk (CS) or wheat straw (WS) supplemented with fibrolytic enzyme on performance, carcass characteristics and meat quality of sheep. For this purpose, thirty male Barki lambs (8 months age, 29 kg b. wt.) were randomly divided into three groups of 10 each. Lambs were fed rations containing 70% concentrate and 30% of either BH, CS or WS. A fibrolytic exogenous enzyme (Fibrozyme®), 2 g/h/day, was added to the ration of animals fed CS or WS. At the end of 10 wks experimental period, there was no difference for efficiency ratio in average daily body weight gain and feed among the groups.

Nagalakshmi and Reddy (2011) carried out an experiment to assess on farm performance of lambs fed expander extruder processed cotton stalks based complete diets. Thirty Nellore ram lambs (4-5month old) of average body weights were randomly divided into two dietary groups. For 180 days, one group was fed an EEP diet containing 38.5 percent CS, while the other was provided a standard diet consisting of concentrates mixture and *ad lib* sorghum stover. The average daily gain was significantly higher ($p<0.01$) of 95g in comparison to 70g when fed conventional ration. The result indicated that cotton stalks could be used as roughage source in complete diets for growing lambs.

Salem *et al.* (2011) evaluated the effect of exogenous enzymes of ZADO® (i.e., ENZ) and on nutrients digestibility and growth performance in six crossbred

sheep (32.00±0.603 kg body weight (BW)) and 6 Baladi goats (18.00±0.703 kg BW) were used in 2 × 2 factorial design. Animals were fed on wheat straw *ad libitum* and restricted amount of commercial concentrate with (+ENZ) or without (-ENZ) 10 g/animal/day of ZADO® to cover 120% of their maintenance requirements. The average daily gain (ADG) was increased ($P<0.05$) in goats with the addition of ENZ (112.8 g/d) as compare with without ENZ (66.7 g/d).

Reddy *et al.* (2012) evaluated growth performance in kids fed expander-extruded complete feed pellets containing red gram (*Cajanus cajan*) straw. Thirty-two weaned male kids of Osmanabadi goat breed in the age group of 4 to 5 months were divided into 4 groups of eight animals each to evaluate two iso-nitrogenous complete mash feeds in which red gram straw was incorporated at 35 and 50% level maintaining total roughage content in both the ration at 60% level. The remaining component of the forage portion comprised of Lucaenea leaves. The experimental feeds (T_1: mash with 35% red gram straw (RGS), T_2: mash with 50% RGS, T_3: pellet with 35% RGS, T_4: Pellets with 50% RGS) were randomly assigned to four treatment groups and animals in the respective groups were offered those feeds for 150 days. The average daily gain under T_1, T_2, T_3 and T_4 was 53.17, 44.12, 73.17 and 69.42 g, respectively. Average daily gain (ADG) in kids was also significantly ($p<0.001$) influenced by feed processing, while the effect of level of inclusion of RGS was non-significant on growth rate.

Torres *et al.* (2013) evaluated an enzymatic extract from *Cellulomonas flavigena* at 0, 2.5, 7.5, 12.5 mL/kg DM of total mixed ration (TMR) on the *in vitro* degradation of DM, NDF and ADF and *in vivo* at 0, 5.0 and 7.5 mL of extract per kg DM of TMR to determine the digestibility and productive performance of lambs fed a TMR made up of 60% forage. Twenty-four Pelibuey-Kathadin lambs were used in the trial. The average body weight and daily gain (ADG) was found to be non-significant ($p>0.05$) in 0.0 ml/kg DM (32.4 kg, and 220 g/d), 5.0 ml/kg DM (32.6 kg and 216 g/d) and 7.5 ml/kg DM of TMR (32.0 kg and 209 g/d), respectively.

Islam *et al.* (2017) evaluated the effect of total mixed ration (TMR) pellet feeding on growth parameters of sheep. Six Bangladeshi Garole sheep (*Ovisaries*)

were randomly divided into two groups (BW: 8±0.5 kg; Age: 1yr). The control diet consisted of roadside grass, rice straw, wheat bran, mustard oil cake, molasses, and common salt, whereas the treatment diet consisted of a pelleted form (P-TMR) and the loose total mixed ration (L-TMR). The sheeps were provided 1.5 times their maintenance energy and protein requirements in both dietary treatments. The result showed that P-TMR had a higher live body weight and better feed conversion ratio ($p<0.05$) than L-TMR.

Kholif *et al*. (2017)[b] demonstrated in a study on three sets of 12 Assaf lambs (4 each) feed fed control, which comprised of 15% alfalfa hay and 85% concentrate feed mixture, (CFM) (on DM basis).; (T_1): Control + 2 g fibrolytic enzymes/h/day; and (T_2): Control + 4 g fibrolytic enzymes/h/day. The growth experiment went on for 28 weeks. Lambs were fed diets at levels of 3.5 % DM of body weight. Total live body weight gain (kg) and daily live body weight gain (g) have shown no significant ($p<0.05$) impact between control (43.225$\pm$3.12 kg, 237.5$\pm$21.4 g), T_1 (43.575$\pm$2.02 kg, 239.4$\pm$17.0 g) and T_2 (46.325$\pm$1.88 kg, 254.5$\pm$19.8 g), respectively.

Jadhav (2019) studied the utilization of cotton stalk in complete pelleted feed in eighteen growing goats of 4- 6 months of age, divided in three equal groups and fed with three different dietary regimes as T_0 (control), T_1 and T_2 groups. Goats in the control group were fed with pelleted feed comprising 60% gram straw and 40% concentrates, while in T_1 group 25% gram straw was replaced by cotton stalk and in T_2 group 50% gram straw was replaced by cotton stalk. Each ration was maintained in 60:40 roughage to concentrate ratio having 12% CP and 60% TDN. The body weights (kg) did not show significant variation between the T_0 (22.48±0.72), T_1 (23.79±0.80) and T_3 (22.95±0.75) groups, however ADG (g/day) was found to be significantly better in T_1 (133.14±7.75) group than that of T_0 (97.22±6.62) and T_2 (103.51±9.27) groups. He concluded that cotton stalk in complete pelleted feed of growing goats can be effectively utilized upto 50% of total roughage source without any adverse effect on growth.

Kedaree *et al.* (2019) conducted an experiment to evaluate performance of growing goats fed paddy straw supplemented with graded levels of *Thespesia*

populneathe. Twelve crossbred bucks were used in a randomized block design for duration of 4 weeks. The treatments comprised of a Portia tree leaves and paddy straw supplemented with in 15:85, 30:70 and 45:55 for the treatment groups T_1, T_2 and T_3, respectively. Concentrate @ 200 g/buck were offered to meet the nutritional requirement of the experimental animals on a dry matter basis. All animals were allowed *ad-libitum* access to water and mineral lick. Supplementation of straw with Portia tree leaves in T_2 significantly increased daily weight gains 75.00 g ($p<0.05$).

Kewan *et al.* (2019) studied the nutritive utilization of *Moringa oleifera* tree stalks treated with fungi and yeast to replace clover hay in growing lambs. In a complete randomised design, 24 Barki lambs with an average body weight of 20.7$\pm$0.17 kg were used to assess the effects of replacing clover (*Trifolium alexandrinum* L) hay as a traditional basal diet (C) with moringa tree stalks (MS) treated with fungi (*Trichoderma reesei*) (MF) and yeast (*Saccharomyces cervisiae*) (MY) under solid-state fermentation on nitrogen. Depending on their live weight, the lambs were separated into three groups, each with eight lambs. The concentrate feed mixture was the same for all groups and was fed at 2% of live weight with *ad libitum* basal roughage. The average body weight and daily gain increases for the C (44.8 kg, 173 g), MF (40.3 kg, 173 g), and MY (41.2 kg, 146 g) groups, respectively.

Mahrous *et al*. (2019)[a] conducted study to observe the effect of biological and chemical treatments of olive trees by-products on chemical composition, degradability, cell wall constituents, digestibility and nutritive value and its feeding effect on productive performance of growing sheep. Eighteen (½ Finnish Landrace × ½ Rahmani) lambs with average body weight 18.00±0.40 kg and 4 months old were used in this study for 120 days. Lambs were distributed into three groups (6 lambs each) and randomly assigned to three experimental rations. The three respective rations composed of concentrate feed mixture (CFM) + olive trees by-products, the control ration (R_1) contained untreated olive tree by-products; (R_2) treated olive trees by products with EM1 and (R_3) treated olive trees by-products with El-mofeed. The digestibility and nutritive values of

experimental rations were determined using nine adult Ossimi rams. Growth performance with respect to total body weight gain and average daily gain (ADG) were improved by biological (21.2 kg and 168 g) and chemical (18.2 kg and 151 g) treatments as compare to control (14.6 kg and 140 g) group.

Shembekar (2019) studied the effect of utilization of ozone treated cotton stalk in pelleted complete feed of eighteen growing goats (4 to 6 months of age) for 90 days. The goats were randomly allotted to three dietary treatments as T_0, receiving pelleted complete feed made from 60% gram straw as roughage and 40% concentrates, T_1 receiving pelleted feed containing 15% ozone treated cotton stalk (OTCS) and 45% gram straw as roughages along with 40% concentrates while, T_2 group received pelleted complete feed containing 30% OTCS and 30% gram straw and 40% concentrates. The roughage to concentrate ratio was maintained as 60:40 in each group. The body weight (kg) significantly higher in T_1 (23.80±0.84) than T_0 (22.48±0.75) and T_2 group (22.72±0.88). The ADG were found to be 97.22±5.91, 135.97±11.50 and 105.74±9.12 g in T_0, T_1 and T_2 groups, respectively with significantly ($p<0.05$) higher ADG in T_1 group as compared to other groups.

Zhang *et al.* (2019) conducted an experiment in growing lambs to see how feeding pelleted total mixed ration (PTMR) affected fattening lambs' growth, ruminal fermentation characteristics, and gastrointestinal microbiota. A total of 100 crossbred (Dorper sheep × Small-tail Han Sheep, DH) ram lambs were randomly assigned to 10 pens at 120 days of age with identical body weight (BW, 34.9 ± 0.5 kg). The pens were randomly assigned to one of two treatments, each with five replicates. Dietary treatments included PTMR and un-pelleted total mixed ration (UPTMR), both of which had the same dietary elements and nutritional content. PTMR-fed lambs had a higher ADG ($P = 0.003$) than UPTMR-fed lambs.

Burghate (2021) studied the effect of utilization of ozone treated cotton stalk in complete pelleted feed in eighteen growing goats of 4- 6 months of age, divided in three equal groups and fed with three different dietary regimes as T_0 (control), T_1 and T_2 groups. Goats in the control group was fed with pelleted feed

comprising 60% gram straw and 40% concentrates, while in T_1 group 75% gram straw was replaced by ozone treated cotton stalk and in T_2 group 100% gram straw was replaced by ozone cotton stalk supplemented with yeast (*Saccharomyces cervisea*) @ 250g/ton of feed and NSP degrading multienzymes @ 500g/ton of feed. Each ration was maintained in 60:40 roughage to concentrate ratio having 12% CP and 60% TDN. The fortnightly body weight gain did not vary significantly amongst the group, whereas overall average body weight gain of 1.52±0.16 kg was observed in T_0 (control) group, followed by T_1 (1.43±0.12 kg) and T_2 (1.33±0.12 kg). ADG was found to be significantly different in T_0 (101.56±8.24 g/day), T_1 (95.22±6.11 g/day) and T_2 (88.67±6.18 g/day) groups.

Ingle (2021) studied the effect of utilization of cotton stalk in complete pelleted feed in eighteen growing goats of 4- 6 months of age, divided in three equal groups and fed with three different dietary regimes as T_0 (control), T_1 and T_2 groups. Goats in the control group were fed with mash complete feed, while in T1 group fed pelleted feed and in T_2 group fed pelleted feed supplemented with yeast (*Saccharomyces cervisea*) @ 250g/ton of feed and NSP degrading multienzymes @ 500g/ton of feed containing 60% gram straw and 40% concentrate mixture. Each ration was maintained in 60:40 roughage to concentrate ratio having 12% CP and 60% TDN. The overall average body weight gain of 1.25±0.10 kg was observed in control group, followed by T_1 (1.52±0.16 kg) and T_2 (1.74±0.13 kg). The average daily gain (g/day) was significantly ($p<0.01$) higher for T_2 (123.94±6.74) group than T_1 (108.86±8.24) and T_0 group (89.67±5.10).

Thorat (2021) studied the utilization of cotton stalk in complete pelleted feed in eighteen growing goats of 4- 6 months of age, divided in three equal groups and fed with three different dietary regimes as T_0 (control), T_1 and T_2 groups. Goats in the control group were fed with pelleted feed comprising 60% gram straw and 40% concentrates, while in T_1 group 75% gram straw was replaced by cotton stalk and in T_2 group 100% gram straw was replaced by cotton stalk supplemented with yeast (*Saccharomyces cervisea*) @ 250g/ton of feed and NSP degrading multienzymes @ 500g/ton of feed. Each ration was maintained in

60:40 roughage to concentrate ratio having 12% CP and 60% TDN. The average fortnightly body weight gain was non-significant in all groups and found 1.52±0.16 kg in control group, followed by T_1 (1.41±0.14 kg) and T_2 (1.22±0.11 kg). ADG was found to be non-significant in T_0 (101.56±8.24 g/day), T_1 (94.08±6.91 g/day) and T_2 (81.45±5.72 g/day) groups. It was concluded that average daily gain was comparatively better in control group fed gram straw based pelleted complete diet.

Mousa *et al.* (2022)[b] conducted an experiment to evaluate the effects of adding different levels of the combination of fibrolytic enzymes and probiotics (a mixture of bacteria and yeast) on the performance of fattening lambs. Thirty-two male Ossimi lambs (weighing 39 ± 0.24 kg) were divided into four groups randomly (eight animals each). The first group (control ration, G1) was fed on a ration of 60% concentrate feed mixture (CFM), 20% Egyptian clover (EC), and 20% wheat straw (WS). The second (G2), third (G3), and fourth (G4) groups were fed a control ration supplemented with Calfo Care® (contains 2500 IU of cellulase, 1250 IU of xylanase, 4.5×10^{10} CFU of *B. licheniformis*, 5.5×10^{10} CFU of *B. subtilis*, and 2.2×10^{10} CFU of *S. cerevisiae* per kilogram) @ of 0.5, 1, and 2 kg/ton diet of dry matter (DM). Results showed that the total weight gain (kg/head) and daily weight gain (g/head) significantly ($p \leq 0.05$) increased in G2 (16.52 and 183.55), G3 (20.06 and 222.89), and G4 (13.68 and 152.00) rations, respectively, compared with the G1 ration (13.18 kg/head and 146.44 g/head).

2.9 Effect of Complete Feed on Dry Mater Intake and DMI (% Body Weight)

Yadav and Deshmukh (2001) evaluated two complete ration containing wheat straw (CR-1) and spent straw (CR-2) each at 60% level in crossbred sheep. The DCP and TDN contents were 5.35, 56.10 and 7.09, 41.68% for CR-1 and CR-2, respectively. The daily DMI was 0.842 and 0.892 kg for wheat and spent straw fed groups, respectively.

Raut *et al.* (2002) observed the effect of pelleted complete feed containing 60% *Cajanus cajan* straw and 40% concentrate mixture in local goats. The daily

DM intake was found to be 743.94±41.56 g and DMI per100 kg BW was calculated to be 4.20±0.28 kg.

Kirubanath *et al*. (2003) examined the effect of processing cotton straw based complete diet with expander-extruder on performance of crossbred calves. Eighteen crossbred calves (6-9 months, 73.48 6.52 kg) were randomly assigned to two full diets and a conventional diet (6 in each group) for 180 days in a growth trial. The complete diets were prepared with 40% cotton straw, one processed in mash form and the other pelletized using an expander-extruder (EEP). These two full meals were compared to a traditional feeding method in which cotton straw and concentrate mixture were fed separately in a 60:40 ratios. In traditional, mash and EEP the DM consumption (kg) was 3.38, 4.13 and 4.76 kg whereas DMI per 100 kg body weight was 2.98, 3.20 and 3.25 kg, respectively. In comparison to the other two groups, the calves on the EEP full diet consumed more DM ($p<0.01$).

Reddy and Reddy (2003) observed the effect of cotton stalks-based complete diet on growth characteristics in sheep and goats in field conditions. An experiment was undertaken on six Nellore ram lambs and six local male kids (4–5 months old). For 180 days, they were fed an expander extruder pressed pelleted complete feed prepared with cotton stalks as the only source of roughage (40%) and concentrates (60%). The DMI per animal (kg) was 1.38 and 1.21 for lambs and goats which were comparable.

Nagalakshmi and Reddy (2011) carried out an experiment to assess on farm performance of lambs and buffaloes fed expander extruder processed cotton stalks based complete diets. Thirty Nellore ram lambs (4-5month old) of average body weights were randomly divided into two dietary groups. For 180 days, one group was fed an EEP diet containing 38.5 percent CS, while the other was provided a standard diet consisting of concentrates mixture and *ad libitum* sorghum stover. The lambs fed on expander extrusion pelletization diet utilized dry matter (DM) with higher ($p<0.01$) feed efficiency.

Salem *et al.* (2011) evaluated the effect of exogenous enzymes of ZADO® (i.e., ENZ) and on nutrients digestibility and growth performance in six crossbred sheep (32.00±0.603 kg body weight (BW)) and 6 Baladi goats (18.00±0.703 kg BW) were used in 2 × 2 factorial design. Animals were fed on wheat straw *ad libitum* and restricted amount of commercial concentrate with (+ENZ) or without (-ENZ) 10 g/animal/day of ZADO® to cover 120% of their maintenance requirements. The average daily gain (ADG) was non-significant ($p>0.05$) in goats with the addition of ENZ (66.7 g/d) and without ENZ (112.8 g/d).

Torres *et al.* (2013) evaluated an enzymatic extract from *Cellulomonas flavigena* at 0, 2.5, 7.5, 12.5 mL/kg DM of total mixed ration (TMR) on the *in vitro* degradation of DM, NDF and ADF and *in vivo* at 0, 5.0 and 7.5 mL of extract per kg DM of TMR to determine the digestibility and productive performance of lambs fed a TMR made up of 60% forage. Twenty-four Pelibuey-Kathadin lambs were used in the trial. There was no significant effect on DM intake in 0.0 (1134 g), 5.0 (1169 g) and 7.5 ml (1143 g) of per kg DM of TMR.

Kholif *et al*. (2017)[b] studied on three sets of 12 Assaf lambs (4 each) feed having control, which comprised of 15% alfalfa hay and 85% concentrate feed mixture, (CFM) (on DM basis).; (T_1): Control + 2 g fibrolytic enzymes/h/day; and (T_2): Control + 4 g fibrolytic enzymes/h /day. The growth experiment went on for 28 weeks. Lambs were fed diets at levels of 3.5 % DM of body weight. The dry matter intake (DMI) differred non-significantly ($p>0.05$) in control (1.79 g/h/d), T_1 (1.76 g/h/d) and T_2 (1.74 g/h/d) group.

Jadhav (2019) studied the utilization of cotton stalk in complete pelleted feed in eighteen growing goats of 4- 6 months of age, divided in three equal groups and fed with three different dietary regimes as T_0 (control), T_1 and T_2 groups. Goats in the control group were fed with pelleted feed comprising 60% gram straw and 40% concentrates, while in T_1 group 25% gram straw was replaced by cotton stalk and in T_2 group 50% gram straw was replaced by cotton stalk. Each ration was maintained with 60:40 roughage to concentrate ratio having 12% CP and 60% TDN. DMI (percent body weight) did not show significant variation between the T_0 (4.48±0.12), T_1 (4.41±0.10) and T_2 (4.36±0.13) groups.

DMI (g) was found to be significantly better in T_1 (1082.46±37.56) group than that of T_0 (1025.52±33.03) and T_2 (1021.88±38.90) groups.

Mahrous *et al*. (2019)[a] conducted to study the effect of biological and chemical treatments of olive trees by-products on chemical composition, degradability, cell wall constituents, digestibility and nutritive value and its feeding effect on productive performance of growing sheep. Eighteen (½ Finnish Landrace × ½ Rahmani) lambs with average body weight 18.00±0.40 kg and 4 months old were used in this study for 120 days. Lambs were distributed into three similar groups (6 lambs each) and randomly assigned to three experimental rations. The three respective rations composed of concentrate feed mixture (CFM) + olive trees by-products, the control ration (R_1) contained untreated olive tree by-products; (R_2) treated olive trees by products with EM1 and (R_3) treated olive trees by-products with El-mofeed. The digestibility and nutritive values of experimental rations were determined using nine adult Ossimi rams. Dry matter intake (DMI) was non-significant in control (978 g), biological (1079 g) and chemical (1235 g) treatments group.

Shembekar (2019) studied the effect of utilization of ozone treated cotton stalk in pelleted complete feed of eighteen growing goats (4 to 6 months of age) for 90 days. The goats were randomly allotted to three dietary treatments as T_0 receiving pelleted complete feed made from 60% gram straw as roughage and 40% concentrates, T_1 receiving pelleted feed containing 15% ozone treated cotton stalk (OTCS) and 45% gram straw as roughages along with 40% concentrates while, T_2 group received pelleted complete feed containing 30% OTCS and 30% gram straw and 40% concentrates. The roughage to concentrate ratio was maintained as 60:40 in each group. He concluded that, T_1 group (1056.20±37.59 g) had significantly ($p \leq 0.01$) higher DMI than T_2 group (981.78±40.75 g) and followed by T_0 group (1025.50±33.03 g). The % DMI was non-significant in T_0 (4.48±0.12), T_1 (4.30±0.11) and T_2 (4.22±0.13) group.

Burghate (2021) studied the utilization of ozone treated cotton stalk in complete pelleted feed in eighteen growing goats of 4- 6 months of age, divided in three equal groups and fed with three different dietary regimes as T_0 (control), T_1

and T_2 groups. Goats in the control group was fed with pelleted feed comprising 60% gram straw and 40% concentrates, while in T_1 group 75% gram straw was replaced by ozone treated cotton stalk and in T2 group 100% gram straw was replaced by ozone cotton stalk supplemented with yeast (*Saccharomyces cervisea*) @ 250g/ton of feed and NSP degrading multi-enzymes @ 500g/ton of feed. Each ration was maintained in 60:40 roughages to concentrate ratio having 12% CP and 60% TDN. The DMI (percent body weight) was found to be significantly higher for T_0 group (4.23±0.05) than that of T_2 (4.10±0.10) and T_0 (3.94±0.07), however DMI for T_0, T_1 and T_2 groups was found to be 673.85±12.39, 669.63±25.23 and 656.68±19.15 g per day ($p>0.05$)

Ingle (2021) studied the utilization of cotton stalk in complete pelleted feed in eighteen growing goats of 4- 6 months of age, divided in three equal groups and fed with three different dietary regimes as T_0 (control), T_1 and T_2 groups. Goats in the control group was fed with mash complete feed, while in T_1 group fed pelleted feed and in T_2 group fed pelleted feed supplemented with yeast (*Saccharomyces cervisea*) @ 250g/ton of feed and NSP degrading multi-enzymes @ 500g/ton of feed containing 60% gram straw and 40% concentrate mixture. Each ration was maintained in 60:40 roughages to concentrate ratio having 12% CP and 60% TDN. The DMI percent BW was found significantly ($p<0.01$) lower for T_0 (3.75±0.03 kg) group than that of T_1 (4.34±0.05 kg) and comparable with T_2 (3.96±0.15 kg) group and DMI was significantly ($p<0.01$) higher in T_2group (694.32±18.22 g/d) as compared to T_0 (620.12±19.22 g/d) and T_1 (673.85±12.39 g/d) groups.

Thorat (2021) studied the effect of utilization of cotton stalk in complete pelleted feed in eighteen growing goats of 4-6 months of age, divided in three equal groups and fed with three different dietary regimes as T_0 (control), T_1 and T_2 groups. Goats in the control group were fed with pelleted feed comprising 60% gram straw and 40% concentrates, while in T_1 group 75% gram straw was replaced by cotton stalk and in T_2 group 100% gram straw was replaced by cotton stalksupplemented with yeast (*Saccharomyces cervisea*) @ 250g/ton of feed and NSP degrading multi-enzymes @ 500g/ton of feed. Each ration was maintained in

60:40 roughages to concentrate ratio having 12% CP and 60% TDN. DMI (percent body weight) showed significant ($p<0.01$) variation between the T_0 (4.23 kg), T_1 (3.60 kg) and T_2 (4.18 kg) groups. DMI was found to be significantly higher for T_0 group (4.23±0.05) than that of T_1 (3.60±0.06) and T_2 (4.18±0.08) and comparable for T_0 and T_1 groups.

2.10 Effect of Complete Feed on Feed Conversion Efficiency

Reddy and Reddy (2003) investigated the effect of a cotton stalks-based complete diet on growth characteristics in sheep and goats in field conditions. A trial was undertaken on six Nellore ram lambs and six local male kids (4–5 months old) for 180 days, and fed an expander extruder pressed pelleted complete feed formulated with cotton stalks as the only source of roughage (40%) and concentrates (60%). The feed conversion efficiency was 7.5 and 8.3 kg in sheep and goats which showed 10.6% higher DMI/kg gain in goats.

Salem *et al.* (2011) evaluated the effect of exogenous enzymes of ZADO® (i.e., ENZ) and on nutrients digestibility and growth performance in six crossbred sheep (32.00±0.603 kg body weight (BW)) and 6 Baladi goats (18.00±0.703 kg BW) were used in 2×2 factorial design. Animals were fed on wheat straw ad libitum and restricted amount of commercial concentrate with (+ENZ) or without (-ENZ) 10 g/animal/day of ZADO® to cover 120 % of their maintenance requirements. Feeding efficiency (kg feed/kg body weight) was significantly improved ($p<0.05$) for ENZ addition (5.6) groups compared to control of without ENZ (9.5) group.

Torres *et al.* (2013) evaluated an enzymatic extract from *Cellulomonas flavigena* at 0, 2.5, 7.5, 12.5 mL/kg DM of total mixed ration (TMR) on the *in vitro* degradation of DM, NDF and ADF and *in vivo* at 0, 5.0 and 7.5 mL of extract per kg DM of TMR to determine the digestibility and productive performance of lambs fed a TMR made up of 60% forage. Twenty-four Pelibuey-Kathadin lambs were used in the trial. There was no significant effect on feed conversion in 0.0 (5.22), 5.0 ml (5.61) and 7.5 ml (5.67).

Jadhav (2019) studied the utilization of cotton stalk in complete pelleted feed in eighteen growing goats of 4- 6 months of age, divided in three equal groups and fed with three different dietary regimes as T_0 (control), T_1 and T_2 groups. Goats in the control group were fed with pelleted feed comprising 60% gram straw and 40% concentrates, while in T_1 group 25% gram straw was replaced by cotton stalk and in T_2 group 50% gram straw was replaced by cotton stalk. Each ration was maintained in 60:40 roughages to concentrate ratio having 12% CP and 60% TDN. He concluded that FCR was found to be significantly better in T_1 (8.80±0.42) group than that of T_0 (12.20± 0.84) followed by T_2 (13.29±1.69) groups.

Kewan *et al.* (2019) to measure growth performance, the experiment was done out on gotas. In a complete randomised design, 24 Barki lambs with an average body weight of 20.7 0.17 kg were used to assess the effects of replacing clover (*Trifolium alexandrinum L*) hay with moringa tree stalks (MS) treated with fungi (*Trichoderma reesei*) (MF) and yeast (*Saccharomyces cervisiae*) (MY) under solid-state fermentation as a traditional basal diet (C) on nitrogen. Depending on their live weight, the lambs were separated into three groups, each with eight lambs. The concentrate feed mixture was the same for all groups and was administered at 2% of live weight with *ad libitum* basal roughage. Feed conversion efficiency found to be highest in MF (7.77) followed by MY (7.22) as compare to control. MY diet recorded the highest ($p<0.05$) feed efficiency compared with MF and control diet (6.20).

Mahrous *et al.* (2019)[a] conducted an experiment to study the effect of biological and chemical treatments of olive trees by-products on chemical composition, degradability, cell wall constituents, digestibility and nutritive value and its feeding effect on productive performance of growing sheep. Eighteen (½ Finnish Landrace × ½ Rahmani) lambs with average body weight 18.00±0.40 kg and 4 months old were used in this study for 120 days. Lambs were distributed into three similar groups (6 lambs each) and randomly assigned to three experimental rations. The three respective rations composed of concentrate feed mixture (CFM) + olive trees by-products, the control ration (R_1) contained

untreated olive tree by-products; (R_2) treated olive trees by-products with EM1 and (R_3) treated olive trees by-products with El-mofeed. The digestibility and nutritive values of experimental rations were determined using nine adult Ossimi rams. Feed conversion as kg DMI/kg gain 6.13, 7.01 and 8.08 for R_2, R_3 and R_1, respectively. The best feed conversion was that for lambs fed R_2.

Shembekar (2019) studied the effect of utilization of ozone treated cotton stalk in pelleted complete feed of eighteen growing goats (4 to 6 months of age) for 90 days. The goats were randomly allotted to three dietary treatments as T_0, receiving pelleted complete feed made from 60% gram straw as roughage and 40% concentrates, T_1 receiving pelleted feed containing 15% ozone treated cotton stalk (OTCS) and 45% gram straw as roughages along with 40% concentrates while, T_2 group received pelleted complete feed containing 30% OTCS and 30% gram straw and 40% concentrates. The FCR was significantly ($p \leq 0.01$) better in T_1 group (8.22±0.35) as compared to T_0 (12.20±0.85) and T_2 (11.39±0.84) groups. He concluded that utilization of OTCS at 25% of total roughage source in pelleted complete feed proved to be more economical for rearing of growing goats under stall fed condition.

Burghate (2021) conducted study on the utilization of ozone treated cotton stalk in complete pelleted feed in eighteen growing goats of 4- 6 months of age, divided in three equal groups and fed with three different dietary regimes as T_0 (control), T_1 and T_2 groups. Goats in the control group was fed with pelleted feed comprising 60% gram straw and 40% concentrates, while in T_1 group 75% gram straw was replaced by ozone treated cotton stalk and in T_2 group 100% gram straw was replaced by ozone cotton stalk supplemented with yeast (*Saccharomyces cervisea*) @ 250g/ton of feed and NSP degrading multienzymes @ 500g/ton of feed. The FCR were found significantly ($p<0.01$) better for T_0 group (8.98±0.73) than that of T_2 (7.07±0.92), however it was comparable with T_1 (11.59±1.16).

Ingle (2021) studied the utilization of cotton stalk in complete pelleted feed in eighteen growing goats of 4-6 months of age, divided in three equal groups and fed with three different dietary regimes as T_0 (control), T_1 and T_2

groups. Goats in the control group was fed with mash complete feed, while in T_1 group fed pelleted feed and in T_2 group fed pelleted feed supplemented with yeast (*Saccharomyces cervisea*) @ 250g/ton of feed and NSP degrading multienzymes @ 500g/ton of feed containing 60% gram straw and 40% concentrate mixture. The FCR was better for T_2 group (7.69±0.67) than that of T_1 (8.98±0.73) and T_0 (9.27±0.55) groups

Thorat (2021) studied the utilization of cotton stalk in complete pelleted feed in eighteen growing goats of 4-6 months of age, divided in three equal groups and fed with three different dietary regimes as T_0 (control), T_1 and T_2 groups. Goats in the control group was fed with pelleted feed comprising 60% gram straw and 40% concentrates, while in T_1 group 75% gram straw was replaced by cotton stalk and in T_2 group 100% gram straw was replaced by cotton stalk supplemented with yeast (*Saccharomyces cervisea*) @ 250g/ton of feed and NSP degrading multienzymes @ 500g/ton of feed. Each ration was maintained in 60:40 roughages to concentrate ratio having 12% CP and 60% TDN. He concluded that FCR were found to be significantly better for T_0 group (8.98±0.73) than that of $T_{2,}$ (11.83±0.61) however it was comparable with T_1 group (10.45±0.95).

2.11 Effect of Complete Feed on Rumen Fermentation Profile

Raut *et al.* (2002) evaluated the pelleted complete feed comprised of 60% *Cajanus cajan* straw and 40% concentrate mixture on local non-descript male goats. The rumen liquor pH on 0, 7, 14, 21 and 28th day of experiment was 7.03, 6.83, 6.83, 6.98 and 6.75, respectively, whereas the NH_3-N and TVFA concentrations in SRL on the respective days were 8.76, 13.33, 14.06, 13.01 and 13.37 mg/100 ml SRL and 4.01, 6.61, 8.74, 8.81 and 8.23 mEq/100 ml SRL, respectively.

Thirumalesh *et al.* (2003) studied the effect of feeding bajra straw based complete diet on performance of adult sheep in 3×3 Latin square design (LSD). The sheep were fed experimental diets T_1 (control) containing 40% ground bajra straw and 60% concentrate mixture separately, T_2 complete diet (pellet) and T_3

complete diet (mash). The non-significant differences were observed for pH (values were 6.38, 6.65 and 6.58, under T_1, T_2 and T_3, respectively). The concentration of NH_3-N was 10.40, 08.71 and 9.24 mg/100 ml at 6 hrs and TVFA concentrations in the rumen fluid were non-significant ($p>0.05$) among different period of rumen liquor collection. The concentrations of Total-N (for T_1, T_2, and T_3 were 103, 118 and 89.50 mg/100 ml) differed significantly ($p>0.05$). All nitrogen fractions (at 0, 2, 4 and 6 h.) were studied and the values for Total-N were 107.33, 93.55, 101.78 and 112.22 mg/100 ml, respectively. For TCA-insoluble protein N the values were 18.00, 16.22, 17.70 and 19.19 mg/100 ml. The result indicated that bajra straw can be incorporated in complete diet of sheep as a sole source of roughage at 40% level.

Gado *et al.* (2007) conducted to study the effect of biological treatments on poor quality roughage (bagasse) to improve the performance of small ruminants. Bagasse ensiled or not using one of the following treatments was: (1) untreated control, (2) with additive mixture solution, (3) cellulase enzyme 15%, (4) cellulase enzyme 25%, (5) rumen liquor, and (6) *Cellulomonas cellulasea.* Two experimental trials were conducted, Experiment (1): goats weighing about 27 kg (±0.5kg) were fed one of the treated bagasse silage groups plus corn (1, 2,3,4,5 and 6). Experiment (2): Twenty male goats (18 kg average initial weight) were used in this experiment. Animal performance (ADG, feed intake and feed efficiency) was calculated each week through-out the experimental period. Results indicated that rumen liquor pH values did not differ significantly ($P>0.05$) among treatments. The values of rumen liquor pH were also similar among times for goats fed biological treated bagasse. The total volatile fatty acids values for treated bagasse by cellulase enzyme (E), rumen liquor (R) and Cellulomonas (B) were higher than that for untreated bagasse (U). The values of sampling time on ammonia N concentration were at the minimum at 0 hrs before feeding and increased to its maximum levels at 4hrs after feeding then values tended to decrease gradually as the time passed up to 6 h after feeding.

Ganai *et al.* (2011) evaluated the effects of exogenous fibrolytic enzymes supplemented to bajra straw @ 0, 1, 2, 3 and 4 g/kg substrate dry matter on

degradability of DM and NDF in T_0, T_1, T_2, T_3 and T_4 group using goat rumen liquor in first experiment. In second experiment, *in vivo* trial was performed to evaluate the effects of exogenous fibrolytic enzymes (optimum level of enzyme 2 g/kg DM selected from experiment I) on rumen fermentation characteristics in complete feed containing bajra straw 60 parts and concentrate mixture 40 parts. There was significant ($p<0.01$) improvement in total volatile fatty acids (84.43 mEq/L) compared to control (81.22 mEq/L). Enzyme application did not alter rumen pH (6.80, 6.84), total nitrogen (93.14, 92.16 mg/dl) as well as ammonia nitrogen (32.89, 32.94 mg/L) in rumen fluid of enzyme supplemented and un-supplemented (control) complete feed, respectively.

Kholif and Aziz (2014) investigated the effects of adding cellulytic enzyme "Asperozym" or Tomoko® to the diets on the performance of goats. Six Baladi lactating goats after 42 days of parturition were assigned randomly into three groups of two animals each using 3x3 Latin square design. The experimental periods were 90 days and consisted of three equal periods. The first group was fed on 50% concentrate feed mixture (CFM), 10% ground date stone and 40% berseem clover (control diet). The second group was fed control diet (C) supplemented with Asperozym at 3.08 U/kg DM (T_1). The third group was fed control diet supplemented with Tomoko® at 1.54 U/kg DM. (T_2). Rumen liquor parameters were significantly ($p<0.05$) affected pH (6.71, 6.59 and 6.67), TVFAs, mg/dl (10.81, 13.36 and 11.57), Ammonia-N, mg/dl (45.92, 55.33 and 48.18), NPN, mg/dl (54.20, 63.98 and 56.93) and Total nitrogen (146.03, 189.50 and 163.35 mg/dl) in control, T_1 and T_2 group.

Torres *et al.* (2013) evaluated an enzymatic extract from *Cellulomonas flavigena* at 0, 2.5, 7.5, 12.5 mL/kg DM of total mixed ration (TMR) on the *in vitro* degradation of DM, NDF and ADF and *in vivo* at 0, 5.0 and 7.5 mL of extract per kg DM of TMR to determine the digestibility and productive performance of lambs fed a TMR made up of 60% forage. The pH of ruminal fluid was found to be 7.0, 6.8 and 6.9 and the total VFA concentration was linearly ($p<0.001$) increased (37.4, 46.0, 56.7 mmol/L) in groups with 0.0 ml/Kg DM, 5.0 ml/kg DM and 7.5 ml/kg DM of TMR, respectively.

Patel *et al.* (2015) revealed the effect of fibrolytic enzyme supplementation on nutrients utilization of sheep was studied. Twelve male Patanwadi sheep (12-15 month) were randomly divided in two groups of 6 each. Sheep were fed rations containing 50 % wheat straw and 50% compound concentrate mixture. A fibrolytic exogenous enzyme @ 0.025% was added to the ration of animal fed TMR. Three animals of each group were used for rumen fermentation study. There was no significant effect on pH (6.64±0.11 and 6.57±0.19), TVFA (118±9.57 and 124±11.88 mEq/L), NH_3-N (9.24±1.56 and 9.26±1.09 mg/dl) and NPN concentrations (70.00±20.58 and 69.69±10.01 mg/dl) however, the Total-N (82.48±16.89 and 88.36±15.15 mg/dl) and protein-N (14.03±2.57 and 18.42±5.36 mg/dl) concentration were significantly ($p<0.05$) higher in treatment group as compared to control group.

Hassan and Almaamory (2016) studied the effect of treatment of source of roughages which are alfalfa hay (AH) and wheat straw (WS) with two source of exogenous fibrolytic enzymes a local enzyme product (LEP) and commercial enzyme product (CEP) on rumen fermentations and some parameters of the blood. Twenty-four Awassi lambs with average initial weight of 29.84 ± 1.37 kg and 9 months were individually fed in a 2×3 factorial experiment. The lambs were randomly divided into six groups according to the type of diet. The diets were: concentrated diet + 8 ml LEP pre-treated WS (T_1), concentrated diet + 8 gm CEP pre-treated WS (T_2), concentrated diet + untreated WS (T3), concentrated diet + 8 ml LEP pre-treated AH (T_4), concentrated diet + 8 gm CEP pre-treated AH (T_5) and concentrated diet + untreated AH (T_6). The results showed that NH_3-N of source of roughages (28.79 and 28.50 mg/dl), enzyme (29.54 and 28.08 mg/dl) and their interaction and pH of source of roughages (6.71 and 6.78), enzyme (6.76 and 6.83) were not affected. It was also observed that treatment of roughages with exogenous fibrolytic enzyme decreased the molar proportions of acetic acid and increased the molar proportions of propionic acid, butyric acid as compared with control.

Kholif *et al*. (2017)[b] studied on three sets of Assaf lambs (4 each) feed having control, which comprised of 15% alfalfa hay and 85% concentrate feed

mixture, (CFM) (on DM basis). (T_1): Control + 2 g fibrolytic enzymes/h/day; and (T_2): Control + 4 g fibrolytic enzymes/h /day. The growth experiment went on for 28 weeks. Lambs were fed diets at levels of 3.5 % DM of body weight. All ruminal pH values were above 6.0. Time of sampling had a significant impact ($p<0.05$) on rumen pH values. TVFA concentration ad NH_3-N demonstrated significant ($p<0.05$) higher values in fibrolytic enzymes supplemented-dietsT_1 (8.45, 13.18, 10.81 meq/dl and 16.64, 25.02, 20.93 mg/dl) and T_2 (9.05, 14.49, 11.50 meq/dl and 16.99, 25.71, 21.06 mg/dl) compared with control (7.24, 11.23, 9.78 meq/dl and 13.01, 22.38, 18.72 mg/dl) group in zero, 3 h and 6 h feeding, respectively. Time of sampling had a significant impact ($p<0.05$) on rumen NH_3-N with an opposite trend of pH values.

Jadhav (2019) studied the utilization of cotton stalk in complete pelleted feed in eighteen growing goats of 4- 6 months of age, divided in three equal groups and fed with three different dietary regimes as T_0 (control), T_1 and T_2 groups. Goats in the control group was fed with pelleted feed comprising 60% gram straw and 40% concentrates, while in T_1 group 25% gram straw was replaced by cotton stalk and in T_2 group 50% gram straw was replaced by cotton stalk. There was no significant variation in pH (6.59±0.02, 6.62±0.02 and 6.62 ±0.02), NH_3-N (17.41 ±0.42, 16.75±0.47 and 16.41±0.41 mg/dl SRL), Total-N (79.91±0.65, 79.62 ±0.73 and 78.45 ±0.66 mg/dl SRL), TCA-ppt-N (42.80 ±0.58, 43.60 ±0.54 and 42.51 ±0.64 mg/dl SRL) and NPN (37.11 ±0.57, 36.45 ±0.82 and 35.94±0.98 mg/dl SRL) in T_0, T_1 and T_2 respectively, whereas TVFA production was significantly higher in T_1 (102.04±2.12 mEq/L SRL) group as compared to T_2 (98.95±1.89 mEq/L SRL) and T_0 (97.58±1.58 mEq/L SRL). He concluded that cotton stalk in complete pelleted feed of growing goats can be effectively utilized upto 50% of total roughage source without any adverse effect on rumen fermentation of goats.

Mahrous *et al.* (2019)[a] conducted an experiment to study the effect of biological and chemical treatments of olive trees by-products on chemical composition, degradability, cell wall constituents, digestibility and nutritive value and its feeding effect on productive performance of growing sheep. Eighteen (½

Finnish Landrace × ½ Rahmani) lambs with average body weight 18.00±0.40 kg and 4 months old were used in this study for 120 days. Lambs were distributed into three similar groups (6 lambs each) and randomly assigned to three experimental rations. The three respective rations composed of concentrate feed mixture (CFM) + olive trees by-products, the control ration (R_1) contained untreated olive tree by-products; (R_2) treated olive trees by products with EM1 and (R_3) treated olive trees by-products with El-mofeed. The digestibility and nutritive values of experimental rations were determined using nine adult Ossimi rams. Concentrations of rumen parameters, NH_3-N (20.95, 28.01 and 24.39 mg/100 ml) and TVFA (12.6, 17.60 and 14.78 meq/100 ml) were significantly ($p<0.05$) increased in R1, R2 and R3 groups, respectively.

Marwan *et al.* (2019) studied to assess the impact of supplementing diets with Exogenous Fibrolytic Enzymes (EFE) in buffalo calves on growth performance criteria, rumen fermentation properties and certain biochemical indices. The total number of 12 buffalo calves of (4-6) months age and 123.3 kg average body weight were assigned into two similar groups (6 animals each). Animals of T_1 (control) were fed on basal ration and those of T_2 (treated) were fed as T_1 plus 12 ml Zymogen liquid (ZL)/100 kg of body weight per head daily. The study was extended for fifteen weeks. Live body weights were individually recorded biweekly in both groups. Rumen fluid samples were gathered from each animal in both groups at the finishing of study for determination of rumen fermentations properties. Results showed that treated group recorded non-significant difference ($p\leq0.05$) in pH 6.93 (treated), 6.83 (control) and significant increase ($p\leq0.05$) in TVFA's (86.97mmol/L) and ammonia concentration (106.91 mmol/L) in comparing with control (80.06 mmol/L and 100.37 mmol/L) group, respectively.

Shembekar (2019) studied the effect of utilization of ozone treated cotton stalk in pelleted complete feed of eighteen growing goats (4 to 6 months of age) for 90 days. The goats were randomly allotted to three dietary treatments as T_0, receiving pelleted complete feed made from 60% gram straw as roughage and 40% concentrates, T_1 received pelleted feed containing 15% ozone treated cotton

stalk (OTCS) and 45% gram straw as roughages along with 40% concentrates while, T_2 group received pelleted complete feed containing 30% OTCS and 30% gram straw and 40% concentrates. There were significant ($p \leq 0.01$) variations in rumen TVFA (9.76±0.16, 10.09±0.24 and 10.17±0.21mEq/dl SRL), NH_3-N (17.41±0.43, 16.08±0.48 and 16.91±0.45 mg/dl SRL) and total nitrogen (79.91±0.65, 82.68±0.69 and 79.91±0.77 mg/dl SRL) in T_0, T_1 and T_2 groups while, rumen pH (6.59±0.025, 6.61±0.027 and 6.62±0.028), TCA-ppt-N (42.80±0.59, 43.45±0.58 and 42.21±0.52 mg/dl SRL) and NPN (37.11±0.57, 39.22±0.84 and 37.69±0.83 mg/dl SRL) concentrations were comparable in T_0, T_1 and T_2 groups. He concluded that, OTCS can be utilized up to 50% of the roughage source in pelleted complete feed of goats without any adverse effect on health and ruminal ecosystem.

Almaamory *et al.* (2020) conducted study on fifteen Awassi male lambs of 4–5 months-old average BW (22±2.2 kg) were used to evaluate the effects of exogenous enzyme (Labazyme and Bio SB-Gold) addition on concentrate diet in the rate of (0. 5g /h/d) intake. The lambs were housed in individual cages to study the effect of exogenous enzymes on the rate of weight gain, rumen fermentation and some blood characteristics. The study showed non-significant differences in rumen fermentation parameters like pH (6.88±0.12, 6.72±0.07 and 6.97±0.11), NH_3-N (27.82±0.85, 28.33±1.01 and 28.33±0.45 mg/dl), TVFA (123.47±1.10, 124.81±0.44 and 125.46±1.79 mmol/l) in control, Labazyme and Bio SB-Gold supplement group, respectively.

Burghate (2021) conducted a study on the utilization of ozone treated cotton stalk in complete pelleted feed in eighteen growing goats of 4- 6 months of age, divided in three equal groups and fed with three different dietary regimes as T_0 (control), T_1 and T_2 groups. Goats in the control group were fed with pelleted feed comprising 60% gram straw and 40% concentrates, while in T_1 group 75% gram straw was replaced by ozone treated cotton stalk and in T_2 group 100% gram straw was replaced by ozone cotton stalk supplemented with yeast (*Saccharomyces cervisea*) @ 250g/ton of feed and NSP degrading multienzymes @ 500g/ton of feed. Each ration was maintained in 60:40 roughages to

concentrate ratio having 12% CP and 60% TDN. The rumen liquor study in rumen pH (6.39±0.08, 6.59±0.06 and 6.48±0.07), TVFA (11.46±0.21, 12.73±0.47 and 13.05±0.44 mEq/100 ml SRL) and NH_3-N (21.03±0.84, 18.94±0.83 and 19.73±0.77 mg/100 ml SRL) varied significantly ($p<0.01$) while, TCA-ppt-N (42.50±1.73, 44.42±0.95 and 43.46±1.06 mg/100 ml SRL), total nitrogen (77.63±1.71, 77.71±1.36 and 78.75±1.71 mg/100 ml SRL) and NPN (35.13±2.14, 33.29±1.72 and 35.29±1.55 mg/100 ml SRL) concentrations were non-significant in T_0, T_1 and T_2 groups, respectively.

Li *et al.* (2021) revealed the effects of feeding pelleted TMR to fattening lambs on feed intake behaviour, growth performance, feed digestion, rumen fermentation characteristics, rumen microbial community, serum parameters, slaughter performance, meat quality, and the economic outcome. Two physical forms (pelleted vs. un-pelleted) of TMR composed of the same ingredients with the same particle sizes were compared in three animal experiments. Experiment 1 having 24 three-month-old healthy uncastrated male F2 hybrids of thin-tailed sheep and Northeast fine-wool sheep with a live weight of 26.3 ± 3.1 kg were used for the experiment. Results indicated that decreased rumen pH in pelleted (6.04) as compare to un-pelleted (6.36) TMR and ammonia nitrogen was not affected in pelleted feed (17.0 mg/dl) and un-pelleted feed (18.4 mg/dl) fed after 3 h morning feeding for lambs.

Ingle (2021) studied the utilization of cotton stalk in complete pelleted feed in eighteen growing goats of 4-6 months of age, divided in three equal groups and fed with three different dietary regimes as T_0 (control), T_1 and T_2 groups. Goats in the control group was fed with mash complete feed, while in T_1 group fed pelleted feed and in T_2 group fed pelleted feed supplemented with yeast (*Saccharomyces cervisea*) @ 250g/ton of feed and NSP degrading multienzymes @ 500g/ton of feed containing 60% gram straw and 40% concentrate mixture. The rumen liquor profile study showed significant variations in rumen TVFA (12.9±0.19, 11.46±0.21 and 12.65±0.30 mEq/100 ml SRL), NH_3-N (22.17±0.72, 21.03±0.84 and 20.14±0.52 mg/100 mlSRL)and total nitrogen (75.67±1.19, 77.63±1.71 and 80.13±1.36 mg/100 ml SRL), TCA-ppt-N(40.29±1.27,

42.50±1.73 and 45.67±1.45 mg/100 ml SRL) in T_0, T_1 and T_2 group, respectively while, rumen pH (6.45±0.06, 6.39±0.08 and 6.50±0.07) and NPN (35.37±2.14, 35.13±2.14 and 34.46±1.72 mg/100 ml SRL) concentration did not vary significantly in T_0, T_1 and T_2 groups.

Santoso *et al.* (2021) studied the nutrient digestion and ruminal fermentation of goats on a complete feed block (CFB) that incorporated agro-industrial by-products and were high in fibre and cellulolytic bacteria. Three Kacang goats, a native Indonesian breed, were used in a 3 × 3 Latin square experimental design with i) CFB without microbes (control), ii) CFB containing 1% *Pseudomonas aeruginosa* and 1% *Acinetobacter baumannii*, and iii) CFB containing 2% *Pseudomonas aeruginosa* and 2% *Acinetobacter baumannii*. Microbes in the CFBs consisted of lactic acid bacteria, yeast and cellulolytic bacteria that ranged from 10^6 to 10^8cfu/g. The goats were fed each day at 8 hrs and 16 hrs. The rumen pH values, propionate and butyrate of goats fed on CFB were not different ($p>0.05$) in control and treatment groups. The addition of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* at 2% level increased ($p<0.05$) ruminal ammonia nitrogen (85.5 mg/dl), acetate (71.3 mM) and TVFA (99.4 mM).

Thorat (2021) studied the utilization of cotton stalk in complete pelleted feed in eighteen growing goats of 4-6 months of age, divided in three equal groups and fed with three different dietary regimes as T_0 (control), T_1 and T_2 groups. Goats in the control group were fed with pelleted feed comprising 60% gram straw and 40% concentrates, while in T_1 group 75% gram straw was replaced by cotton stalk and in T_2 group 100% gram straw was replaced by cotton stalk supplemented with yeast (*Saccharomyces cervisea*) @ 250g/ton of feed and NSP degrading multi-enzymes @ 500g/ton of feed. The rumen liquor profile revealed non-significant ($p\leq0.01$) variations in rumen TVFA (11.46±0.21, 11.52±0.37 and 11.24±0.26 mEq/100 ml SRL), NH_3-N (21.03±0.84, 22.38±0.45 and 22.11±0.63 mg/100 ml SRL), total nitrogen (77.63±1.71, 75.67±1.75 and 82.21±2.38 mg/100 ml SRL), TCA-ppt-N (42.50±1.73, 44.00±1.00 and 44.67±1.44 mg/100 ml SRL) and NPN (35.13±2.14, 31.67±2.27 and 38.00±2.38

mg/100 ml SRL) in T_0, T_1 and T_2 groups respectively while, rumen pH (6.39±0.08, 6.53±0.07 and 6.63±0.07) in T_0, T_1 and T_2 group was comparable.

2.12 Effect of Complete Feed on Digestibility

Rajmane and Deshmukh (2000) tested three full meals consisting 60 percent sorghum straw (C1), soyabean straw (C2), and maize cobs (C3) each with 40 percent concentrate combination on fifteen adult non-pregnant goats of the Osmanabadi breed. They divided the goats into three groups. The digestibility of DM, CP, CF, EE and NFE under C1, C2 and C3 was 47.31 ± 2.06, 60.16 ± 1.75 and 64.81 ± 2.78; 79.60 ± 4.26, 81.04 ± 2.01 and 85.63 ± 2.76; 49.29 ± 3.10, 58.84 ± 1.37 and 67.38 ± 3.4; 79.50 ± 2.09, 76.28 ± 2.68 and 46.81 ± 6.59 & 45.49 ± 1.41, 58.11 ± 3.45 and 63.72 ± 2.73, respectively. The digestibility of DM, NFE ($p<0.01$) and CF ($p<0.05$) was higher on CR-3 compared to C1 while the digestibility of EE was higher ($p<0.01$) on C1 compared to C3. The CP digestibility did not vary among the groups.

Belewu and Adeniyi (2001) determined the effects of consumption of fungus treated cotton waste by growing goats on feed intake and digestibility. Nine West African dwarf goats (7.5 ± 0.65 kg average trial BW) in a 3 x 3 Latin square with 21d periods, consumed cotton seed cake diet (diet A), cotton seed meal (diet B) and fungus treated cotton waste (diet C) *ad libitum*. Dry matter and crude protein digestibility increased more for diet C ($p<0.05$) than the mean for diets A and B. Crude fibre digestibility ranked ($p < 0.05$) B (70.41%) > A (65.7%) >C (58.20%) and ADF digestibility was 50.25, 60.66 and 74.20% (± 6.75 SE). NDF was greatest ($p<0.05$) for diet C, greater ($P<0.05$) for diet B than for diet A (67.70, 58.84 and 42.20%, respectively).

Yadav and Deshmukh (2001) evaluated two complete ration containing wheat straw (CR-1) and spent straw (CR-2) each at 60% level in crossbred sheep. The DCP and TDN contents were 5.35, 56.10 and 7.09, 41.68% for CR-1 and CR-2, respectively. They reported that digestibility of DM, NFE, NDF and ADF was significantly higher on C1 than in C2 group.

Raut *et al.* (2002) conducted a trial on local non-descript male goats to evaluate the pelleted complete feed containing 60% *Cajanus cajan* straw and 40% concentrate mixture (jowar 30, cotton seed cake 27, arhar chunni 30, groundnut cake 10, mineral mixture 2 and common salt 1%). The values for DCP and TDN contents of the complete feed were 8.95 and 55.12%, respectively. The digestibility coefficients were 56.43 (DM), 66.37 (CP), 74.96 (EE), 67.56 (CF), 45.64 (NFE), 48.95 (NDF) and 40.44 (ADF).

Hamza *et al.* (2005) carried out a study to observe the effect of incubated cotton stalks and rice straw with white rot fungi growth (*Pleurotus ostreatus*) on the digestibility (*in vitro* and *in vivo*) and feeding value of cotton stalks and rice straw. Results showed that digestibility coefficients of DM (63.68, 65.05 and 58.42, 55.73%%), OM (70.15, 70.26 and 63.38, 61.36%), CP (69.30, 67.54 and 60.00, 64.60%) and NFE (75.08, 79.53 and 67.12, 68.7%) were significantly higher ($p<0.05$) in ration contained treated rice straw, cotton stalks than untreated rice straw, cotton stalks, respectively. Feeding value for TDN (58.71, 58.82 and 54.91, 51.94%) and DCP % (8.32, 10.41 and 6.50, 9.83%) was improved as a result of biological treatment than untreated one. It was concluded that biological treatment could be used successfully to enrich the poor quality roughages and improved digestibility coefficient and feeding value of treated materials.

Azzaz *et al.* (2012) conducted an experiment to evaluate the effect of cellulases addition to banana wastes on dry matter (IVDMD) and organic matter (IVOMD) disappearances. Laboratory produced cellulase (Asperozym) and a commercial cellulolytic enzyme source (Bacillozym®) were added separately to banana wastes at 4 levels (0, 0.77, 1.54, 2.31 and 3.08 Unit/kg DM). Increasing the Asperozym levels up to 3.08 U/kg DM exhibited the highest ($p<0.05$) IVDMD and IVOMD, while Bacillozym® recorded the highest ($p<0.05$) IVDMD and IVOMD values at 1.54 U/kg DM compared with the untreated banana wastes (Control). Nine lactating Zaraibi goats (about 3 years old and weight on average 31±0.2 kg) after parturition were divided into three groups of three animals each, using 3x3 Latin square designs to evaluate the effect of Asperozym and Bacillozym® addition to diets on the productivity of lactating goats. Animals

were fed on 50% Concentrate Feed Mixture (CFM), 25% banana wastes and 25% berseem (clover) straw (control diet). Control diet+ Asperozym at level of 3.08 U/kg DM (T_1); control diet+Bacillozym® at level of 1.54 U/kg DM. (T_2). Nutrient digestibility for all nutrients were improved ($p<0.05$) by cellulases treatments group as compared to control group.

Patel *et al.* (2015) revealed the effect of fibrolytic enzyme supplementation on nutrients utilization by sheep. Twelve male Patanwadi sheep (12-15 month) were randomly divided in two groups of 6 each. Sheep were fed rations containing 50 % wheat straw and 50% compound concentrate mixture. A fibrolytic exogenous enzyme @ 0.025% was added to the ration of animal fed TMR. At the end of 14th week experimental period, animals were kept on digestion trial and three animals of each group were used for rumen fermentation analysis. The digestibility % of CP (78.14±1.06 and 84.27±1.14) and NFE (62.25±0.47 and 64.99±0.58) were observed significantly ($p<0.05$) higher in fibrolytic exogenous enzyme @ 0.025% fed TMR group than without enzyme fed TMR group.

Aboul-Fotouh *et al.* (2017) conducted by using nine of lactating Baladi goats and divided into three groups, three animals per each group. The first group was fed 50% concentrate feed mixture, 10% Egyptian clover and 40% wheat straw (Control ration). The second group was fed control ration supplemented with Asperozym (locally produced cellulase enzyme) at level of 1000 unit of cellulase enzyme /kg DM intake (R1). The third group was fed control ration supplemented with Phytabex plus ® (commercial cellulolytic enzyme source) at level of 1000 unit of cellulolytic enzymes /kg DM intake (R2). Rations supplemented with Asperozym (R1) and Phytabex plus® (R2) significantly ($P\leq0.05$) increased DM, OM, CF and NFE digestibility compared to the control ration and non-significant differences were found between Asperozym and phytabex plus rations concerning CP and EE digestibilties. Digestible crude protein (7.67, 7.23%) and total digestible nutrients (68.74, 67.33%) of rations supplemented with cellulolytic enzymes were insignificantly higher in R1 and R2 than control (7.13 %, 65.13%) ration, respectively.

Hassan and Almaamory (2019) studied the effect of treatment of two source of roughages Alfalfa hay (AH) and wheat straw (WS) with two source of exogenous fibrolytic enzymes (EFE): local enzyme product (LEP) and commercial enzyme product (CEP) on growth performance, Feed Conversion Ratio and Nutrient Digestibility. Twenty-four Awassi lambs were randomly divided into six groups according to the type of diet. Concentrated diet + 8 ml LEP pre-treated WS (T_1), concentrated diet + 8 gm CEP pre-treated WS (T_2), concentrated diet + untreated WS (T_3), concentrated diet + 8 ml LEP pre-treated AH (T_4), concentrated diet + 8 gm CEP pre-treated AH (T_5) and concentrated diet + untreated AH (T_6). The results showed that the dry matter, organic matter, crude fiber, NDF, ADF, ADL digestibility's were not affected by the source of enzyme or the source of roughages and their interaction. While, the crude protein, ether extract, and hemicelluloses digestibilities were decreased ($p<0.01$) with interaction between CEP and WS.

Jadhav (2019) studied the utilization of cotton stalk in complete pelleted feed in eighteen growing goats of 4- 6 months of age, divided in three equal groups and fed with three different dietary regimes as T_0 (control), T_1 and T_2 groups. Goats in the control group was fed with pelleted feed comprising 60% gram straw and 40% concentrates, while in T_1 group 25% gram straw was replaced by cotton stalk and in T_2 group 50% gram straw was replaced by cotton stalk. There were no significant differences in DM (58.15 ±0.53, 57.99 ±0.51 and 57.87 ±0.48), CP (68.32 ±1.23, 69.62 ±0.44 and 68.80 ±0.74), EE (79.43 ±0.60, 81.60 ±0.85 and 80.77 ±0.69) and ADF (28.49 ±0.99, 31.87 ±0.98 and 29.88 ±0.75) between T_0, T_1 and T_2 group, respectively. However, CF (58.82±1.05, 59.60±0.67), NFE (56.93±0.75, 54.82±0.58), NDF (39.30±0.75, 39.84±0.88), Hemicellulose (65.34±1.12, 60.87±1.14) and Cellulose (77.87±0.50, 76.20±0.60) digestibility was found to be significantly better in T_0 and T_1 groups than that of T_2 (53.51±0.91, 49.19±0.32, 36.50±0.55, 56.34±1.22 and 73.59±0.29) group, respectively. He concluded that cotton stalk in complete pelleted feed of growing goats can be effectively utilized upto 50% of total roughage source without any adverse effect on digestibility of nutrients.

Shembekar (2019) studied the effect of utilization of ozone treated cotton stalk in pelleted complete feed of eighteen growing goats (4 to 6 months of age) for 90 days. The goats were randomly allotted to three dietary treatments as T_0, receiving pelleted complete feed made from 60% gram straw as roughage and 40% concentrates, T_1 receiving pelleted feed containing 15% ozone treated cotton stalk (OTCS) and 45% gram straw as roughages along with 40% concentrates while, T_2 group received pelleted complete feed containing 30% OTCS and 30% gram straw and 40% concentrates. The roughage to concentrate ratio was maintained as 60:40 in each group. He concluded that the digestibility coefficients for CP (68.32±1.23, 69.79±0.61 and 66.52±1.02), EE (79.43±0.60, 79.32±0.60 and 78.87±0.40), CF (58.83±1.05, 58.61±0.99 and 54.62±1.58), NFE (56.93±0.75, 54.87±1.03 and 54.91±0.78), NDF (39.31±1.85, 38.02±1.22 and 35.85±1.01). ADF (28.49±0.99, 29.81±1.29 and 26.36±1.05) and hemicellulose (65.35±1.12, 61.65±1.77 and 63.60±1.73) were non-significant in T_0, T_1 and T_2, respectively. Different nutrients except ($p \leq 0.05$) DM (58.12±0.53, 57.37±0.74 and 55.02±0.85) and cellulose (77.87±0.51, 77.18±0.63 and 75.47±0.36) were non-significant in T_0, T_1 and T_2, respectively.

Malik *et al.* (2020) carried out an experiment to assess the effect of physical form and level of wheat straw inclusion on growth performance and blood metabolites of fattening goat. Thirty-two male Beetal goats (27.4 ± 0.28 kg body weight (BW)) were divided randomly into four dietary treatments with a 2 × 2 factorial arrangements (n=8/treatment): (1) CTMR15 (conventional TMR containing 15% WS), (2) CTMR25 (conventional TMR containing 25% WS), (3) PTMR15 (pelleted TMR containing 15% WS), and (4) PTMR25 (pelleted TMR containing 25% WS). DM, OM, CP, NDF, ADF and EE digestibilities were similar ($p>0.05$) in all treatments. Nitrogen balance, nitrogen intake, faecal nitrogen, urinary nitrogen, and retained nitrogen were also not influenced ($P>0.05$) by the physical form or the straw level in the diet.

Li *et al.* (2021) studied the effects of feeding pelleted TMR to fattening lambs on feed intake behaviour, growth performance, feed digestion, rumen fermentation characteristics, rumen microbial community, serum parameters,

slaughter performance, meat quality, and the economic outcome. Two physical forms (pelleted vs. un-pelleted) of TMR composed of the same ingredients with the same particle sizes were compared in three animal experiments. 24 three-month-old healthy uncastrated male F2 hybrids of thin-tailed sheep and Northeast fine-wool sheep with a live weight of 26.3 ± 3.1 kg were used in the experiment 1. The digestibility of organic matter (66.0, 66.1%), crude protein (70.2, 72.0 %), neutral detergent fibre (50.2, 50.5%), acid detergent fibre (28.4, 30.4%), and ether extract (92.5,94.3%) were not affected in pelleted and un-pelleted TMR, respectively and dry matter digestibility was slightly lower in pelleted (61.6%) as compared to un-pelleted TMR (64.7%).

Santoso *et al.* (2021) added cellulolytic bacteria in complete feed block based on agro-industrial by products for Kacang goats. Three Kacang goats, a native Indonesian breed, were used in a 3 × 3 Latin square experimental design with i) CFB without microbes (control), ii) CFB containing 1% *Pseudomonas aeruginosa* and 1% *Acinetobacter baumannii*, and iii) CFB containing 2% *Pseudomonas aeruginosa* and 2% *Acinetobacter baumannii*. Microbes in the CFBs consisted of lactic acid bacteria, yeast and cellulolytic bacteria that ranged from 10^6 to 10^8cfu/g. Goats fed on CFB with microbes 1% and 2% had higher ($p<0.01$) digestibility of organic matter (60.2, 62.00 %) and NDF (45.9, 48.6%) compared with control (58.2, 42.2%).

Burghate (2021) conducted study on the utilization of ozone treated cotton stalk in complete pelleted feed in eighteen growing goats of 4- 6 months of age, divided in three equal groups and fed with three different dietary regimes as T_0 (control), T_1 and T_2 groups. Goats in the control group were fed with pelleted feed comprising 60% gram straw and 40% concentrates, while in T_1 group 75% gram straw was replaced by ozone treated cotton stalk and in T_2 group 100% gram straw was replaced by ozone cotton stalk supplemented with yeast (*Saccharomyces cervisea*) @ 250g/ton of feed and NSP degrading multienzymes @ 500g/ton of feed. The nutrient digestibility (%) of DM (64.80±1.49, 63.22±1.61 and 62.99±2.44), EE (77.71±1.93, 78.56±0.76 and 76.11±0.42), CF (62.80±4.72, 60.16±2.27 and 57.49±1.81), NFE (68.33±2.72, 65.31±1.56 and

62.93±1.60), NDF (39.78±2.36, 36.95±1.14 and 39.04±0.91), ADF (33.09±1.06, 33.82±1.09 and 34.87±0.87), hemicellulose (64.91±1.57, 63.85±1.05 and 64.35±1.42) and cellulose (73.07±1.24, 71.43±0.68 and 71.10±0.51) were non-significant in T_0, T_1 and T_2 groups, respectively and significant ($p<0.01$) difference in CP amongst all the dietary T_0 (71.34±0.78), T_1 (71.61±0.67) and T_2 (67.82±0.82) groups was observed. He concluded the beneficial effect of ozone treatment of cotton stalk for 75 percent replacement and even complete replacement of gram straw in pelleted complete feed of goats.

Ingle (2021) studied the effect of utilization of cotton stalk in complete pelleted feed in eighteen growing goats of 4- 6 months of age, divided in three equal groups and fed with three different dietary regimes as T_0 (control), T_1 and T_2 groups. Goats in the control group were fed with mash complete feed, while in T_1 group fed pelleted feed and in T_2 group fed pelleted feed supplemented with yeast (*Saccharomyces cervisea*) @ 250g/ton of feed and NSP degrading multienzymes @ 500g/ton of feed. Each ration was maintained 60:40 roughages to concentrate ratio having 12% CP and 60% TDN. The DM and CP digestibility of T_2 (71.26±1.59 and 73.01±1.01) group was significantly ($p<0.01$) higher than the T_0 (54.79±2.90 and 60.46±1.62) group, however it was comparable with T_1 (64.80±1.49 and 71.34±0.78) group. EE (72.09±0.97, 77.71±1.93 and 79.33±1.01), NDF (37.70±0.45, 39.78±2.36 and 45.08±1.95), ADF (30.53±0.60, 33.09±1.06 and 35.47±1.38), hemicellulose (60.80±1.58, 64.91±1.57 and 69.13±1.97), cellulose (63.71±1.87, 73.07±1.24 and 74.43±0.44) vary significantly within dietary treatment groups T_0, T_1 and T_2 and CF (53.51±4.04, 62.80±4.72 and 66.07±3.12) and NFE (61.08±3.43, 68.33±2.72 and 69.51±2.42) digestibility did not vary significantly within dietary treatment groups T_0, T_1 and T_2, respectively.

Thorat (2021) studied the utilization of cotton stalk in complete pelleted feed in eighteen growing goats of 4-6 months of age, divided in three equal groups and fed with three different dietary regimes as T_0 (control), T_1 and T_2 groups. Goats in the control group were fed with pelleted feed comprising 60% gram straw and 40% concentrates, while in T_1 group 75% gram straw was

replaced by cotton stalk and in T_2 group 100% gram straw was replaced by cotton stalk supplemented with yeast (*Saccharomyces cervisea*) @ 250g/ton of feed and NSP degrading multienzymes @ 500g/ton of feed. The nutrient digestibility (%) of DM (64.80±1.49, 61.57±2.44 and 58.55±1.877), CP (71.34±0.78, 69.52±2.84 and 65.71±2.25), EE (77.71±1.93, 74.70±3.31 and 72.41±2.15), CF (62.80±4.72, 58.33±1.52 and 58.66±2.33), NFE (68.33±2.72, 65.78±1.33 and 61.06±2.32), NDF (39.78±2.36, 37.93±0.97 and 39.72±0.78), ADF (33.09±1.06, 35.26±0.84 and 35.24±1.30), hemicellulose (64.91±1.57, 66.90±1.63 and 65.45±0.94) and cellulose (73.07±1.24, 74.00±0.64 and 73.41±1.04) were non-significant amongst all the dietary treatment T_0, T_1 and T_2 groups. The research indicated the beneficial effect of cotton stalk for 75 percent replacement and even complete replacement of gram straw in pelleted complete feed of goats.

Mousa *et al.* (2022)[b] conducted to evaluate the effects of adding different levels of the combination of fibrolytic enzymes and probiotics (a mixture of bacteria and yeast) on the performance of fattening lambs. Thirty-two male Ossimi lambs (weighing 39 ± 0.24 kg) were divided into four groups randomly (eight animals each). The first group (control ration, G1) was fed on a ration of 60% concentrate feed mixture (CFM), 20% Egyptian clover (EC), and 20% wheat straw (WS). The second (G2), third (G3), and fourth (G4) groups were fed a control ration supplemented with Calfo Care® (contains 2500 IU of cellulase, 1250 IU of xylanase, 4.5×10^{10} CFU of *B. licheniformis*, 5.5×10^{10} CFU of *B. subtilis*, and 2.2×10^{10} CFU of *S. cerevisiae* per kilogram) @ of 0.5, 1, and 2 kg/ton diet of dry matter (DM). Results showed that significant ($p \leq 0.05$) increased in the DM, organic matter, crude protein, crude fiber, and ether extract digestibility in G2 (62.79, 66.67, 63.44, 62.74 and 75.57 %) and G3 (65.39, 68.80, 65.24, 66.70 and 77.08 %) rations was observed compared with the G1 (57.41, 62.45, 57.41, 57.51 and 63.24 %) and G4 (58.55, 63.88, 56.62,58.90 and 59.51%) rations, respectively. Nitrogen free extract digestibility percentage was non-significant ($p > 0.05$) in G1 (63.44), G2 (67.50), G3 (68.50) and G4 (64.62) ration. Moreover, the percentages of total digestible nutrients (TDN) and digestible crude protein (DCP) increased ($p \leq 0.05$) in G2 (62.67 and 9.20 %) and G3 (64.07 and

9.46 %) rations compared with the G1 (57.37 and 8.32 %) and G4 (57.48 and 8.21 %) rations.

2.13 Effect of Complete Feed on Blood Biochemical's

Pi *et al.* (2005) conducted to evaluate the effect of pre-treatment and pelletization on nutritive value of rice straw-based total mixed ration (TMR) (Trial 1), and to investigate growth performance, carcass traits and meat quality of growing Boer goats fed on the TMR. Thirty-six growing Boer goats were used in Trial 2. They were divided into three equal groups and randomly allocated to receive one of the following diets: (1) unpelletized ryegrass hay-based TMR (TMR-1), (2) pre-treated pelletized-TMR in Trial 1 (TMR-2), and (3) TMR-2 plus 0.12% zinc-methionine (TMR-3). The experimental period lasted 75 days with the first 15 days for adaptation. On the final day, blood samples were collected to measure serum biochemical parameters. Concentrations of total protein and albumin were significantly higher in the serum of goats fed on TMR-2 (71.7 g/l and 56.9 g/l) and TMR-3 (77.8 g/l and 60.6 g/l) than those on TMR-1 (63.00 g/l and 50.00 g/l) ($p < 0.05$) while globulin was significantly lower in TMR-1 (13.0 g/l) and TMR-2 (14.8 g/l) than TMR-3 (17.2 g/l). Blood urea nitrogen was significantly lower ($P<0.05$) in goats fed on TMR-2 (7.3 mmol/l) and TMR-3 (7.3 mmol/l) than those on TMR-1 (8.6 mmol/l).

Gado *et al.* (2007) conducted an experiment to study the effect of biological treatments on poor quality roughage (bagasse) to improve the performance of small ruminants. Bagasse ensiled or not using one of the following treatments: (1) untreated control, (2) with additive mixture solution, (3) cellulase enzyme 15%, (4) cellulase enzyme 25%, (5) rumen liquor, and (6) Cellulomonas cellulasea. The results of the study indicated that plasma total protein increased between 0 & 6 hrs in all treatments except bagasse treated by solution but this increasing under normal determination of plasma total protein in goats. After 21 days of feeding on treated and untreated bagasse, urea concentration increased gradually from 0 hrs until 6 hrs in all treatments. Also, it is significant values between treated bagasse.

Azzaz *et al.* (2012) conducted an experiment to evaluate the effect of cellulases addition to banana wastes on dry matter (IVDMD) and organic matter (IVOMD) disappearances. Laboratory produced cellulase (Asperozym) and a commercial cellulolytic enzyme source (Bacillozym®) was added separately to banana wastes at 4 levels (0, 0.77, 1.54, 2.31 and 3.08 Unit/kg DM). Increasing the Asperozym levels up to 3.08 U/kg DM exhibited the highest ($p<0.05$) IVDMD and IVOMD, while Bacillozym® recorded the highest ($p<0.05$) IVDMD and IVOMD values at 1.54 U/kg DM compared with the untreated banana wastes (Control). Nine lactating Zaraibi goats (about 3 years old and weight on average 31±0.2 kg) after parturition were divided into three groups of three animals each, using 3x3 Latin square design to evaluate the effect of Asperozym and Bacillozym® addition to diets on the productivity of lactating goats. Animals were fed on 50% Concentrate Feed Mixture (CFM), 25% banana wastes and 25% berseem (clover) straw (control diet). Control diet+ Asperozym at level of 3.08 U/kg DM (T1); control diet+Bacillozym® at level of 1.54 U/kg DM. (T2). Blood plasma total protein, albumin, globulin and urea concentration were higher ($p<0.05$) in goat fed cellulytic enzymes diet T_1 (6.4, 3.4, 2.9 g/dl and 21.7 mg/dl), T_2 (6.6, 3.5, 3.1 g/dl and 22.8 mg/dl) than control diet (5.8, 3.2, 2.6 g/dl and 21.3 mg/dl) respectively. The addition of Asperozym and Bacillozym® to diets improved the performances of lactating Zaraibi goats with no deleterious effects on general health.

Hassan and Almaamory (2016) studied the effect of treatment of source of roughages as alfalfa hay (AH) and wheat straw (WS) with two source of exogenous fibrolytic enzymes a local enzyme product (LEP) and commercial enzyme product (CEP) on rumen fermentations and some parameters of the blood. Twenty-four Awassi lambs with average initial weight of 29.84 ± 1.37 kg and 9 months were individually fed in a 2×3 factorial experiment. The lambs were randomly divided into six groups according to the type of diet. The diets were: concentrated diet + 8 ml LEP pre-treated WS (T_1), concentrated diet + 8 gm CEP pre-treated WS (T_2), concentrated diet + untreated WS (T_3), concentrated diet + 8 ml LEP pre-treated AH (T_4), concentrated diet + 8 gm CEP pre-treated AH (T_5) and concentrated diet + untreated AH (T_6). Results showed that the blood

parameter, serum urea nitrogen was not affected by the source of enzyme (36.41±3.19 mg/dl) for LEP and (41.58±1.92 mg/dl) for CEP and the source of roughages (41.94±1.75 mg/dl) for AH and (36.83±2.28 mg/dl) for WS compared with control (40.16±2.93 mg/dl).

Rivero *et al.* (2016) investigated the effect of exogenous enzyme of xylanase, *Salix babylonica* extract and their combination on blood haematological and biochemical profile in lambs and goats. Suffolk lambs (4) and Saanen goats (4) were used in a Latin square design (4 animals × 4 treatments in 4 periods) for 15 days of adaptation. Animals were fed the basal diet plus 30 ml of water (control), or plus 30 ml of exogenous enzyme xylanase (EZ), or plus 30 ml of *S. babylonica extract* (SB), or plus 30 ml of xylanase + 30 ml of SB extract (EZSB). The daily dose of treatments was given orally before the morning feeding. Blood samples (5 ml) were collected on day 15 of each period and analyzed for haematological and biochemical profile. All blood parameters, urea (6.7, 7.1, 8.3 and 7.5 mmol/l) in control, EZ, SB and EZSB, respetively were within the normal range for healthy goats and it is concluded that xylanase, *S. babylonica* and their combination did not pose any threat to the health of the animals under the conditions of the experiment where both feed additives are innocuous.

Aboul-Fotouh *et al.* (2017) conducted by using nine of lactating Baladi goats divided into three groups, three animals per each group. The first group was fed 50% concentrate feed mixture, 10% Egyptian clover and 40% wheat straw (Control ration). The second group was fed control ration supplemented with Asperozym(locally produced cellulase enzyme) at level of 1000 unit of cellulase enzyme /kg DM intake (R1). The third group was fed control ration supplemented with Phytabex plus ® (commercial cellulolytic enzyme source) at level of 1000 unit of cellulolytic enzymes /kg DM intake (R2). Results of serum urea analysis showed no side effect of using the tested cellulolytic enzymes on lactating goats.

Kholif *et al*. (2017)[b] studied on three sets of 12 Assaf lambs (4 each) feed having control, which comprised of 15% alfalfa hay and 85% concentrate feed mixture, (CFM) (on DM basis).; (T_1): Control + 2 g fibrolytic enzymes/h/day; and

(T_2): Control + 4 g fibrolytic enzymes/h /day. The growth experiment went on for 28 weeks. Lambs were fed diets at levels of 3.5 % DM of body weight. Blood components: Total protein and globulin contents significantly higher ($p<0.05$) lambs diets with low level of fibrolytic enzymes in T_1 (7.20±0.56, 3.50±0.32 g/dl) than control (6.30±0.47, 2.50±0.28 g/dl) or high fibrolytic enzymes level in T_2 (6.30+0.49, 3.00+0.38 g/dl). Albumin content reduced with high level of enzymes in T_2 (3.30±0.41 g/dl) than the control (3.80±0.44 g/dl) or in T_1 (3.80±0.38) diets. While urea in treated rations did not significantly ($p>0.05$) affect its level 37.00±1.22, 37.33±1.28 mg/dl in T_1 and T_2 as compared to control (35.00±1.07 mg/dl).

Kholf *et al.* (2017)[c] assessed the impacts of fibrolytic enzymes supplementation on milk yield, milk content, blood serum parameters and the feed effectiveness and sparing assessment in lactating Zaraibi goats. Nine lactating Zaraibi goats following 7 days of parturition were isolated into 3 gatherings, 3 animals every, utilizing 3×3 latin square outlines for 84 days. The principal gathering was sustained 37.5% focus nourish blend (CFM), 12.5% date piece and half berseem roughage (control eat less carbs). The second gathering was nourished control eat less carbs supplemented with Veta-Zyme Plus® at level 15 U/kg DM. The 3rd gathering was encouraged control abstain from food supplemented with Asperozyme at level 45 U/Kg DM. Blood serum parameters viz. total protein (g/dl), albumin(g/dl) and urea (mg/dl) were significantly ($p<0.05$) higher in T_2 (7.43, 3.61 and 24.08) followed by T_1 (7.29, 3.57 and 22.63) as compare with control (6.75, 3.08 and 18.42). Serum globulin concentration (g/dl) was non-significant in control (3.67), T_1 (3.71) and T_2 (3.82).

Odusanya *et al.* (2017) carried out a research trial to evaluate the response of West African Dwarf (WAD) rams (n= 20) with average live weight of 13.7±1.16 kg to varying levels of 0, 10, 20 and 30% cassava (*Manihot esculenta*) leaf (CL) meal concentrate diet in a completely randomized design for a period of 90 days. The data were collected on performance, haematology, carcass and meat sensory characteristics. The analysis of blood parameters showed significant ($p<0.05$) differences in values obtained for total protein in 20% CL (10.59g/dl)

and 30% CL (10.90g/dl) as compare to 0 % CL (8.49g/dl) and 10% CL (8.37g/dl). Other parameter, urea was found within normal range (20.00, 18.50, 18.88 and 19.22 g/dl) in 0% CL, 10% CL, 20% CL and 30%CL, respectively for clinically healthy WAD ram.

Chen *et al.* (2018) investigated the effect of intact rapeseed (IR) supplementation in goat total mixed ration (TMR) pellets on performance, carcass traits, serum biochemical indices and meat quality. Forty-eight healthy Boer goats with similar initial bodyweight (12.52 $\pm$ 1.48 kg) were randomly assigned to four dietary treatment groups, containing 0%, 2.5%, 5.0% and 7.5% IR, respectively. The results showed that IR supplementation have non-significant ($p>0.05$) change in serum total protein (67.60$\pm$6.30, 67.28$\pm$1.48, 68.38$\pm$60.3 and 72.85$\pm$50.9 g/L), albumin (34.37+1.46, 33.15+1.74, 32.62+2.89 and 34.88+3.86 g/L), globulin (33.23+4.87, 34.13+2.71, 35.75+5.11 and 37.98+3.39 g/L) and blood urea nitrogen (5.80+0.44, 5.38+0.99, 5.43+0.47 and 5.95+0.94 mmol/L) in 0%, 2.5%, 5.0% and 7.5% IR, respectively.

Al-Salmany *et al.* (2019) carried out a study to assess the effect of exogenous fibrolytic enzyme (EFE) (Safizym) in blood characters of local male kids. The animals were randomly assigned to three treatments (6 animals for each treatment) a control treatment was fed on basal ration without enzyme (T_c), a treated group were supplemented with the EFE of 500 g/ton of concentrate feed (T_1) and a treatment with the EFE of 1000 g/ton of concentrate feed (T_2). The results showed no significant effect of the adding with EFE in the total protein, albumin and globulin, while there was an effect of the period of the weeks in the T_1, ($p\leq0.05$) of the eighth week. The results showed a significant difference ($P\leq0.05$) in the serum urea concentration.

Li Quanwei *et al.* (2019) investigated effect of pelleted total mixed ration on production performance and serum biochemical index of growing Hainan Black goats. Twenty-four growing Hainan Black goats with same genetic backgrounds and similar initial weight of (10.05$\pm$0.049) kg were randomly assigned into two treatments and supplied with Juncao + concentrate (control

group), pelleted total mixed ration (experimental group), respectively. The significant difference in serum biochemical parameters between experimental group and control group ($p>0.05$), total protein (63.70±2.66, 64.57±4.38 g/L), albumin (28.32±1.77, 30.30±1.71 g/L), slightly increased while the globulin (35.38+3.18 g/L, 34.27+2.86 g/L), urea nitrogen (5.50±0.63, 5.08±0.38 mmol/L) were observed in the study.

Mahrous *et al.* (2019)[a] conducted study to observe the effect of biological and chemical treatments of olive trees by-products on chemical composition, degradability, cell wall constituents, digestibility and nutritive value and its feeding effect on productive performance of growing sheep. Eighteen (½ Finnish Landrace × ½ Rahmani) lambs with average body weight 18.00±0.40 kg and 4 months old were used in this study for 120 days. Lambs were distributed into three similar groups (6 lambs each) and randomly assigned to three experimental rations. The three respective rations composed of concentrate feed mixture (CFM) + olive trees by-products, the control ration (R1) contained untreated olive tree by-products; (R2) treated olive trees by-products with EM1 and (R3) treated olive trees by-products with El-mofeed. The results showed that blood serum constituents, total protein (6.43 6.55 and 6.60 g/dl), serum albumin (3.69, 3.79 and 4.01 g/dl), serum globulin (2.74, 2.76 and 2.59 g/dl) and blood urea (43.82, 42.15 and 43.61) mg/dl were generally normal in R1, R2 and R3 experimental groups respectively.

Mahrous *et al.* (2019)[b] conducted research to evaluate changes in productive performance, in terms of milk yield and its composition, of lactating Damascus goats as a result of feeding three rations. The three respective rations composed of concentrate feed mixture (CFM) + olive trees by-products, the control ration (R1) contained untreated olive tree by-products; (R2) treated olive trees by-products with EM1(biological treatment) and (R3) treated olive trees by-products with El-mofeed (91% molasses; 2.5% urea and 6.5% mixing minerals and vitamins). Eighteen lactating Damascus goats aged 24 - 30 months, weighed 37.20±0.2 kg and were in 2nd - 3rd season of lactation were distributed according to their live body weight and milk production into three similar groups, (6 goats

each). The experiment lasted for 120 days after the does weaned their offspring. The results of blood serum parameters showed insignificant ($p>0.05$) values of total protein (6.79, 6.72 and 6.85 g/dl), albumin (3.91, 3.87 and 3.91 g/dl) and globulin (2.88, 2.85 and 2.94 g/dl) and blood urea (51.69, 48.61 and 59.60 mg/dl) concentrations in R1, R2 and R3, respectively.

Marwan *et al.* (2019) studied the impact of supplementing diets with Exogenous Fibrolytic Enzymes (EFE) in buffalo calves on growth performance criteria, rumen fermentation properties and certain biochemical indices. The total number of 12 buffalo calves of (4-6) months age and 123.3 kg average body weight were assigned into two similar groups (6 animals each). Animals of T_1 (control) were fed on basal ration and those of T_2 (treated) were fed as T_1 plus 12 ml Zymogen liquid (ZL)/100 kg of body weight per head daily. The study was extended for fifteen weeks. Live body weights were individually recorded biweekly in both group. The results showed that blood biochemical analysis in treated group (T_2) showed significant increase ($p\leq0.05$) in total protein (6.24 g/dl), albumin (2.26 g/dl) and non-significant decrease in globulin (3.98 g/dl), urea (27.88 mg/dl) levels compared with control group (5.85 g/dl, 1.90g/dl, 3.95g/dl and 28.31 mg/dl), respectively.

Almaamory *et al.* (2020) conducted study on fifteen Awassi male lambs of 4 – 5 months-old average BW (22±2.2 kg) were used to evaluate the effects of exogenous enzyme (Labazyme and Bio SB-Gold) addition on concentrate diet in the rate of (0. 5g /h/d) intake. The lambs were housed in individual cages to study the effect of exogenous enzymes on the rate of weight gain, rumen fermentation and some blood characteristics. The study showed non-significant differences in blood biochemical as serum urea nitrogen (27.00±0.00, 27.00±+0.00 and 27.25±0.25 mg/dl) in control, Labazyme and Bio SB-Gold supplement group.

Sarmin *et al.* (2020) evaluated the hematological and chemical parameters of the blood of female fat tail sheep raised with fermented complete feed (CF) management. Forty adult female fat tail sheep aged between 24-36 months were used and body weight in 30-35 kg and 3-4 of body condition score, which was raised in the individual stall with fermented complete feed (CF) management.

Samples of blood were drawn once from the jugular vein before feeding in the morning. The results of blood biochemical parameter of albumin (3.36±0.31 g/dl). The results of blood biochemical parameters in the study were within normal range as per reference of the adult female fat tail sheep raised with the fermented complete feed (CF) management.

Li *et al.* (2021) studied the effects of feeding pelleted TMR to fattening lambs on feed intake behaviour, growth performance, feed digestion, rumen fermentation characteristics, rumen microbial community, serum parameters, slaughter performance, meat quality, and the economic outcome. Two physical forms (pelleted vs. un-pelleted) of TMR composed of the same ingredients with the same particle sizes were compared in three animal experiments. Experiment 1 having 24 three-month-old healthy uncastrated male F2 hybrids of thin-tailed sheep and Northeast fine-wool sheep with a live weight of 26.3 ± 3.1 kg were used for the experiment. Serum parameters as total protein (75.8, 73.2 g/L), albumin (27.5, 26.8 g/L), globulin (48.3, 46.4 g/L) and BUN (9.13, 7.67 mmol/L) were not affected in pelleted and unpelleted feed.

Mousa *et al.* (2022)[b] conducted trial to evaluate the effects of adding different levels of the combination of fibrolytic enzymes and probiotics (a mixture of bacteria and yeast) on the performance of fattening lambs. Thirty-two male Ossimi lambs (weighing 39 ± 0.24 kg) were divided into four groups randomly (eight animals each). The first group (control ration, G1) was fed on a ration of 60% concentrate feed mixture (CFM), 20% Egyptian clover (EC), and 20% wheat straw (WS). The second (G2), third (G3), and fourth (G4) groups were fed a control ration supplemented with Calfo Care® (contains 2500 IU of cellulase, 1250 IU of xylanase, 4.5×10^{10} CFU of *B. licheniformis*, 5.5×10^{10} CFU of *B. subtilis*, and 2.2×10^{10} CFU of *S. cerevisiae* per kilogram) @ of 0.5, 1, and 2 kg/ton diet of dry matter (DM).Results showed that the total protein and albumin concentrations were significantly ($p \leq 0.05$) increased in G2 (7.34 and 3.82 g/dl), G3 (7.43 and 3.89 g/dl), and G4 (7.45 and 3.84 g/dl) rations compared with the G1 (6.94 and 3.48 g/dl) ration whereas, globulin (g/dl) comparable in G1 (3.47), G2 (3.53), G3 (3.54) and G4 (3.61). Furthermore, rations G2 (29.25), G3 (28.58)

and G3 (28.95) decreased ($p \leq 0.05$) the blood serum urea (mg/dl) concentration compared with G1(33.96).

2.14 Economic of Feeding

Kirubanath *et. al.* (2003) studied the effect of processing cotton straw based complete diet with expander-extruder on performance of crossbred calves. A growth trial was conducted on eighteen crossbred calves (6-9 months, 73.48±6.52 kg) by randomly allotting to two complete diets and a conventional diet (6 in each group) for 180 days. The complete diets were formulated containing 40 per cent cotton straw, one processed in mash form and other subjected to expander-extruder pelletization (EEP). These two complete diets were compared with conventional system of feeding under which concentrate mixture and cotton straw were fed separately in a 60:40 ratio. Expander-extrusion though increased the cost of production, it reduced the cost of feed per unit live weight gain by 12.28% in comparison to its mash form and by 16.76% when concentrate and cotton straw were fed separately. The results showed that combining cotton straw with concentrates in a complete diet improved growth performance and may be used for cost-effective ration to crossbred calves.

Reddy and Reddy (2003) evaluated effect of cotton stalks based complete diet on growth and carcass characteristics in sheep and goats in field condition. A complete diet was formulated with cotton stalks as sole source of roughage (40.0%) and processed into pellets using expander extruder and fed to 6 Nellore rant lambs and 6 local male kids (4–5 m) to record its effect on growth and meat production in sheep and goats. They were fed expander extruder pressed pelleted complete feed formulated with cotton stalks as sole source of roughage (40%) with concentrates (60%) for 180 days. The cost of feed /kg gain in sheep and goat was Rs. 24.3 and 26.9, respectively which show 10.7 % higher cost of feed/kg gain in goats than lambs.

Nagalakshmi and Reddy (2011) studied on farm performance of lambs and buffaloes fed expander extruder processed cotton stalks based complete diets. Thirty Nellore ram lambs (4–5-month-old) of average body weights were

randomly divided into two dietary groups to assess the effect of feeding expander extruder processed complete diet (EEP) containing cotton stalks (CS) as sole roughage source on growth performance. One group was fed with EEP diet containing 38.5% CS and other with conventional diet i.e., concentrates mixture and ad lib sorghum stover, fed separately for 180 days. The cost of feed/kg gain of lambs was lower ($p<0.05$) fed on EEP diet.

Kholif *et al.* (2017)[b] conducted study on three sets of 12 Assaf lambs (4 each) feed having control, which comprised of 15% alfalfa hay and 85% concentrate feed mixture, (CFM) (on DM basis).; (T_1): Control + 2 g fibrolytic enzymes/h/day; and (T_2): Control + 4 g fibrolytic enzymes/h /day. The growth experiment went on for 28 weeks. Lambs were fed diets at levels of 3.5 % DM of body weight. The feed cost/kg gain decreased and economical feed efficiency was higher with fibrolytic enzymes level and was the lower with control.

Jadhav (2019) studied the utilization of cotton stalk in complete pelleted feed in eighteen growing goats of 4-6 months of age, divided in three equal groups and fed with three different dietary regimes as T_0 (control), T_1 and T_2 groups. Goats in the control group was fed with pelleted feed comprising 60% gram straw and 40% concentrates, while in T_1 group 25% gram straw was replaced by cotton stalk and in T_2 group 50% gram straw was replaced by cotton stalk. Each ration was maintained in 60:40 roughage to concentrate ratio having 12% CP and 60% TDN. The cost of feed per kg body weight gain of experimental goats was less in T_1 (Rs. 103.75) group fed with substitution of 25% gram straw by cotton stalk than that of T_0 (Rs. 140.71) and T_2 (Rs.123.81) groups. He concluded that utilization of 25% cotton stalk of total roughage found to be more economical in rearing of goats under stall fed condition.

Kewan *et al.* (2019) studied the effect of utilization of *Moringa oleifera* tree stalks treated with fungi and yeast to replace clover hay in growing lambs to measure growth performance. Twenty-four Barki lambs with an average body weight of 20.7 ± 0.17 kg were used in a complete randomized design to evaluate the effects of replacing clover (*Trifolium alexandrinum* L) hay as a traditional basal diet (C) with moringa tree stalks (MS) treated with fungi (*Trichoderma*

reesei) (MF) and yeast (*Saccharomyces cervisiae*) (MY) under solid-state fermentation on nitrogen and water metabolism, rumen fermentation and economic efficiency of feeds. Lambs were divided into three groups each with eight lambs depending on their live weight. Concentrate feed mixture was similar for all groups and was offered at 2% of live weight with the basal roughage offered *ad libitum*. MY and MF diet recorded the highest ($p<0.05$) economical feed efficiency and Relative economic efficiency % compared with control diet.

Mahrous *et al.* (2019)[a] studied the effect of biological and chemical treatments of olive trees by-products on chemical composition, degradability, cell wall constituents, digestibility and nutritive value and its feeding effect on productive performance of growing sheep. Eighteen lambs with average body weight 18.00±0.40 kg and 4 months old were used in this study for 120 days. Lambs were distributed into three similar groups (6 lambs each) and randomly assigned to three experimental rations. The three respective rations composed of concentrate feed mixture (CFM) + olive trees by-products, the control ration (R_1) contained untreated olive tree by-products; (R_2) treated olive trees byproducts with EM1 and (R_3) treated olive trees by-products with El-mofeed. The results showed that feed cost/kg gain and relative feed cost % were decreased as biological and chemical treatments in tested ration (R_2 and R_3) compared to control ration (R1). Economic feed efficiency increased in biological treatment group (R_2) as compare to control.

Shembekar (2019) worked to study the effect of utilization of ozone treated cotton stalk in pelleted complete feed of eighteen growing goats (4 to 6 months of age) for 90 days. The goats were randomly allotted to three dietary treatments as T_0, receiving pelleted complete feed made from 60% gram straw as roughage and 40% concentrates, T_1 receiving pelleted feed containing 15% ozone treated cotton stalk (OTCS) and 45% gram straw as roughages along with 40% concentrates while, T_2 group received pelleted complete feed containing 30% OTCS and 30% gram straw and 40% concentrates. The roughage to concentrate ratio was maintained as 60:40 in each group. He concluded that Feeding cost per kg body weight gain was found to be Rs. 140.71, 102.90 and 119.10 in T_0, T_1 and

T_2 groups, respectively, indicating that, T_1 treatment was more economical fed pelleted complete feed having15% ozone treated cotton stalk (OTCS) and 45% gram straw as roughages with 40% concentrates.

Li *et al.* (2021) revealed the effects of feeding pelleted TMR to fattening lambs on feed intake behaviour, growth performance, feed digestion, rumen fermentation characteristics, rumen microbial community, serum parameters, slaughter performance, meat quality, and the economic outcome. Two physical forms (pelleted vs. un-pelleted) of TMR composed of the same ingredients with the same particle sizes were compared in three animal experiments. 24 three-month-old healthy uncastrated male F2 hybrids of thin-tailed sheep and Northeast fine-wool sheep with a live weight of 26.3 ± 3.1 kg were used in the experiment 1. Profit per animal was higher from lambs fed pellets than those fed un-pelleted feed.

Burghate (2021) conducted study on the utilization of ozone treated cotton stalk in complete pelleted feed in growing goats divided in three equal groups and fed with three different dietary regimes as T_0 (control), T_1 and T_2 groups. Goats in the control group was fed with pelleted feed comprising 60% gram straw and 40% concentrates, while in T_1 group 75% gram straw was replaced by ozone treated cotton stalk and in T_2 group 100% gram straw was replaced by ozone cotton stalk supplemented with yeast (*Saccharomyces cervisea*) @ 250g/ton of feed and NSP degrading multienzymes @ 500g/ton of feed. The cost of feed per kg body weight gain was lowest in T_2 group (Rs. 91.61) where complete gram straw was replaced with ozone treated cotton stalk followed by T_1 group (Rs. 94.69) where 75 percent gram straw was replaced with ozone treated cotton stalk, however feeding cost was highest inT_0 group (Rs 106.02) fed gram straw based pelleted complete feed.

Ingle (2021) studied the utilization of cotton stalk in complete pelleted feed in eighteen growing goats divided in three groups and fed with three different dietary regimes as T_0 (control), T_1 and T_2 groups. Goats in the control group was fed with mash complete feed, while in T_1 group fed pelleted feed and in T_2 group fed pelleted feed supplemented with yeast (*Saccharomyces cervisea*) @ 250g/ton of feed and NSP degrading multienzymes @ 500g/ton of feed containing 60%

gram straw and 40% concentrate mixture. The cost of feed per kg body weight gain was lowest in T_2 group (Rs. 96.63) where supplementing with fiber degrading enzymes and yeast in pelleted feed followed by T_1 group (Rs. 106.02) and T_0 group (Rs. 116.39).

Thorat (2021) studied the effect of utilization of cotton stalk in complete pelleted feed in growing goats fed on three different dietary regimes as T_0 (control), T_1 and T_2 groups. Goats in the control group was fed with pelleted feed comprising 60% gram straw and 40% concentrates, while in T_1 group 75% gram straw was replaced by cotton stalk and in T_2 group 100% gram straw was replaced by cotton stalk supplemented with yeast (*Saccharomyces cervisea*) @ 250g/ton of feed and NSP degrading multienzymes @ 500g/ton of feed. The cost of feed per kg body weight gain was lowest in T_1 group (Rs. 95.26) where 75 percent gram straw was replaced with cotton stalk followed by T_2 group (Rs. 101.45) and T_0 group (Rs. 106.02). It was concluded from the observations that utilization of 75 percent replacement of gram straw with cotton stalk as roughage source gave maximum profit under intensive system of rearing.

MATERIALS AND METHODS

The present study was undertaken to assess the effect of treatment of cotton stalk with liquid fibrolytic enzymes on *in vitro* digestibility and evaluating the effect of inclusion of treated cotton stalk in pelleted complete feed as a sole source of roughage on growth performance, rumen fermentation, digestibility of nutrients and economics of stall-fed growing goats.

3.1 Place of work

The work related to preparation of liquid fibrolytic enzyme was conducted in the Department of Chemical Engineering, Visvesvaraya National Institute of Technology, Nagpur. While, rest of the experimental work such as standardization of treatment with regard to dose and hour, *in vitro* digestibility and *in vivo* study on experimental goats was carried out at Instructional Livestock Farm Complex and Department of Animal Nutrition, Nagpur Veterinary College, Nagpur.

3.2 Period of experiment

The liquid fibrolytic enzyme was prepared during 01/07/2021 to 30/10/2021. The treatment of liquid fibrolytic enzymes on cotton stalk for standardization of enzymes dose (dilution) and soaking duration was done from 01/11/2021 to 26/02/2022 by conducting *in vitro* dry matter digestibility experiment. After standardization of doses, the treatment of cotton stalk with liquid fibrolytic enzymes and assessment of *in vitro* digestibility was performed for treatment groups from 01/04/2022 to 15/06/2022. For *in vivo* study, feeding trial was conducted from 15/07/2022 to 14/11/2022 on growing goats for 120 days by offering them pelleted complete feed comprising of treated cotton stalk as sole roughage. Before the start of the animal experiment, a two-week acclimatization period was offered to experimental goats.

3.3 Climatic condition

The climatic conditions at Nagpur are harsh. The soil structure in the Nagpur region is medium dark and red, with varying depths and structures. In the summer, the maximum temperature in Nagpur can reach up to 47^0C, while the temperature in the winter can drop to 6^0 Celsius.

3.4 Extraction and purification of enzymes

The basic work of enzymes extraction and purification and enzyme quantification/assay, enzymes thermal stability and enzymes degree of degradation were conducted at Department of Chemical Engineering, Visvesvaraya National Institute of Technology, Nagpur.

3.4.1 Preparation of enzymes

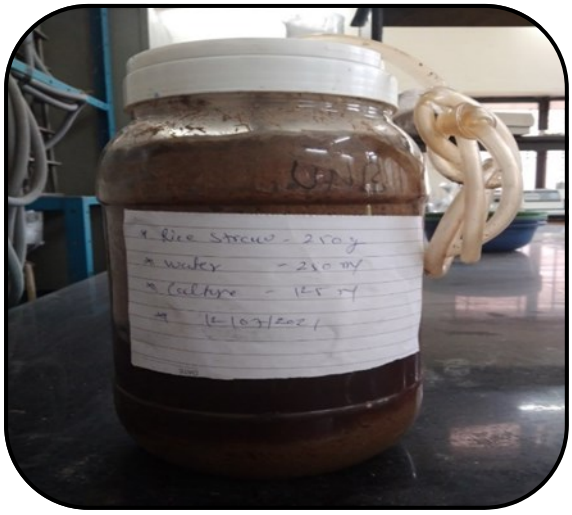
Stage 1. Shredding: Obtained biomass (rice straw) is shredded into a particle size of 1 mm x 2 mm.

Stage 2. Solid State Fermentation: Biomass: Water: Culture = 1:1:0.5, set for fermentation for 14-15 days (residence time).

Stage 3. Buffer Extraction: The buffer used for extraction was the Phosphate buffer (Na_2HPO_4 and NaH_2PO_4) of pH 6, soaked overnight and solid to liquid ratio is 1:10 because the result indicated the most efficient extraction volume and possible reason is solvent inhibition.

Stage 4. Centrifuge: Carried out the centrifuge at 10000 rpm for 15 min at $< 10^{\circ}$C. Centrifuged 4 lit extract per cycle. The purpose of the centrifuge was to lyse the remaining microbial cells in buffer extract.

Stage 5. Membrane Separation/Filtration: Filtration was done by membrane separator MWCO- 75k Da. Operated under 2 bar pressure to prevent damage to the membrane. Backwashing is done to prevent membrane clogging after every extraction process.



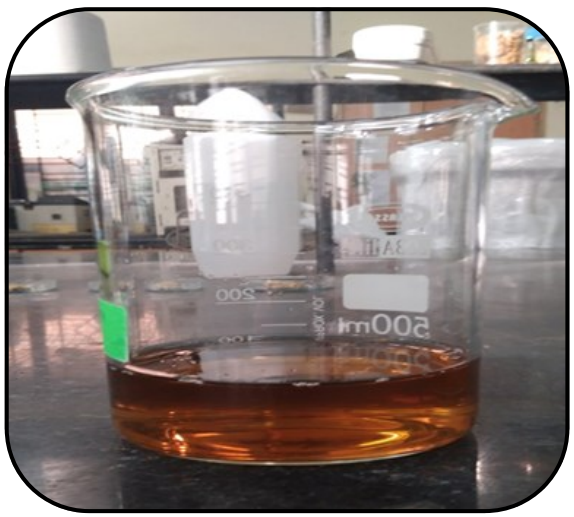
Substrate fermentation

Buffer extraction

Centrifugation

Membrane separation

Rice straw based centrifuged crude liquid enzymes



Membrane separated extract

Plate 3.1 Enzymes extraction and purification

3.4.2 Assay/Quantitative analysis of enzymes

3.4.2 (a) Cellulase Assay-

A total cellulase system consists of three enzymatic activities: endoglucanases, exoglucanases, and β-d-glucosidases. Total cellulase activities are always measured using insoluble substrates, including pure cellulosic substrates such as Whatman No. 1 filter paper, cotton linter, microcrystalline cellulose, bacterial cellulose, and algal cellulose, as well as cellulose-containing substrates such as dyed cellulose, a-cellulose, and pretreated lignocellulose. A cellulose analysis was done as per Filter Paper Assay method (Adney and Baker, 2008). Filter paper activity (FPA) is the most common total cellulase activity assay recommended by the International Union of Pure and Applied Chemistry (IUPAC). IUPAC recommends a FPA assay that differs from most enzyme assays based on the soluble substrate for initial reaction rates.

3.4.2 (b) Xylanase Assay

The xylanase assay was made by DNS method (Miller,1959) and was based on the production of reducing sugar from a solution of xylan and the reducing sugar was then reacted with 3,5 Dinitrosalicyclic Acid (DNS). The said assay was outsourced from Cargill Premix & Nutrition – India.

3.4.3 Degradation degree testing for enzyme

3.4.3. (a) Cellulose Estimation

Chemicals Required

a) Acetic/Nitric Reagent (150 ml of 80% Acetic acid with 15 ml of conc. Nitric acid is mixed)

b) Sulphuric Acid (67%)

c) Anthrone Reagent (200 mg Anthrone+100 ml H_2SO_4)

d) Standard Cellulose Solution (100 mg of Cellulose+10 ml 67% H_2SO_4. 1 ml of this was added to 100 ml H_2O)

Procedure:

A dry sample of 0.5-1.0 g was taken in a dry test tube, and 3 mL of acetic/nitric reagent was added, and the sample was well mixed using a vortex mixture. The solution was placed in a water bath at 100 °C for 30 min. Then the mixture was cooled and centrifuged for 15-20 min at 10,000 rpm, and the supernatant was discarded. The residue was washed with water, and 10 mL of 67% H_2SO_4 was added and kept aside for 1 h. From the solution, 1 ml was taken and diluted to 100 ml, and 1 ml of the diluted solution was then added to 10 ml of anthrone reagent and mixed well. Tubes were heated in a boiling water bath for 10 minutes, and the absorbance was measured at 630 nm by taking a blank with anthrone reagent and water. The amount of cellulose was calculated from the standard graph of cellulose prepared by taking 0.4 to 2 ml of standard cellulose solution (Viles and Silverman, 1949).

3.4.3. (b) Hemicellulose Estimation

Chemicals Required

Sulphuric Acid - 1%

Anthrone Reagent (200 mg Anthrone+100 ml H_2SO_4)

Procedure

For hemicellulose estimation, dry lignocellulosic biomass was treated with 1% sulphuric acid, at 100°C for 4 h. Then, the treated lignocellulosic biomass was dried overnight. Hemicellulose content was determined from the total soluble sugar content difference between control and treated lignocellulosic biomass (Marlett and Lee, 2006).

3.4.4 Enzyme thermal stability

A 100 ml of dilute enzyme was taken in a wide petri plate and was subjected to drying under vacuum at different temperatures, ranging from 40°C, 50°C and 60°C. Treatment duration was fixed at 6 hours and samples were collected at the end of the drying cycle. The concentrated samples thus obtained were then applied on to virgin rice straw and incubated for a period of 4 days. Degree of degradation was then determined using the Anthrone method for measurement of cellulose and hemicellulose content. Similar experiment was done with the dilute enzyme cocktail being subjected to a rotary evaporator. The vacuum was intermittently applied while the temperature was maintained constant at 40°C. The treatment was carried out until the final volume reached one tenth of the initial value. This was done as an observation experiment to evaluate the extent upto which concentration of enzyme is possible.

3.5 *In vitro* digestibility studies

The final liquid fibrolytic enzyme product was evaluated for the standardization at Nagpur Veterinary College, Nagpur, in terms of dose (dilution) and time (duration) of treatment of cotton stalk by conducting *in vitro* dry matter digestibility.

3.5.1 Treatment on Cotton Stalk and standardization study

As per the optimization study, extracted and purified rice straw substrate-based liquid enzymes were mixed with equal quantity of water to form the enzyme solution used for the treatment. The shredded cotton stalk was treated by incubating with enzymes solution 8 L/kg (1:8) of the cotton stalk for 24, 48, 72 and 96 h. Also, the cotton stalk was treated with enzyme solution by incubating @ 4 L/kg (1:4) of the shredded cotton stalk for 24, 48, 72 and 96 h with the treatment of shredded cotton stalk with 8 and 4 L of enzyme solution more leachate (water extract) was obtained. So, various combination for reduction in volume of enzyme solution were tried and accordingly. The treatment on shredded cotton stalk was done by spraying with enzyme solution @ 250 ml/kg of the cotton stalk and

incubating it for 4, 6 and 8 h and also with @ 500 ml/kg of the cotton stalk for 4, 6 and 8 h. Also, treatment was done by spraying water equal to volume of enzyme solution on shredded cotton stalk @ 250 ml/kg and @ 500 ml/kg cotton stalk for 4, 6 and 8 h incubation.

3.5.2 Collection of Rumen Liquor

Rumen liquor was withdrawn from the rumen of the goat using a smooth plastic stomach tube 2 h post feeding. Rumen liquor was immediately transported to the laboratory in an insulated jug with a temperature maintained at 39^0 C and then filtered by four layers of muslin cloth.

3.5.3 Preparation of Phosphate Carbonate Buffer solution

The Phosphate Carbonate Buffer solution (artificial saliva) was prepared according to McDougall (1948) as

a.	$NaHCO_3$	9.80g
b.	$Na_2HPO_42H_2O$	7.00g
c.	KCL	0.57g
d.	NaCl	0.47g
e.	$MgSO_42H_2O$	0.12g
f.	$CaCl_2$	0.04g

Mixing the above chemicals except $CaCl_2$, which was added just before use, was kept temperature at 39°C and CO_2 was passed through a solution.

3.5.4 *In vitro* digestibility determination

In vitro digestibility was determined, taking 0.5 g dried and ground (particle size < 1mm) shredded cotton stalk of each treated with enzymes solution in 100ml conical flask for the first stage *in vitro* method (Tilley and Terry, 1963) along with 40 ml phosphate carbonated buffer (McDougall, 1948), 10 ml of strained rumen liquor (SRL) with flushing of CO_2 gas and incubated for 48 h with periodically shaking at 39°C and parallel blank (without sample) with phosphate carbonated buffer and rumen liquor without substrate. Anaerobic conditions were created in the system by bubbling CO_2 gas, maintaining pH to 6.8, and after 48 hr

Spraying with enzyme solution

Incubation

Drying after treatment

Plate 3.2 Treatment of shredded cotton stalk with spraying of enzyme solution, incubation and drying for *in vitro* study

Sample of cotton stalk for *in vitro* digestibility

Passing CO_2 gas in phosphate carbonate buffer

Passing CO_2 gas in sample containing strain rumen liquor

Shaking incubator for *in vitro* digestibility

Plate 3.3 *In vitro* digestibility

incubation, filter the contents through Sintered Glass Crucible (G1). The residue is dried at 100°C overnight and used to estimate % IVDMD.

Calculations:

DM disappearance= Wt. of sample – (wt. of residue of test – wt. of residue of blank)

$$\text{DM digestibility (\%)} = \frac{\text{DM disappearance x 100}}{\text{Wt. of sample (DM basis)}}$$

3.6 Proximate analysis

The proximate composition of various ingredients and pellets was determined as per AOAC (2005) standard methods.

3.6.1 Dry Matter (DM)

The moisture content of the sample was determined by heating it to a constant weight in an oven at 100^0-105^0 Celsius under air pressure. Dry matter was the consistent weight of a sample after it had been completely dehydrated.

$$\text{Moisture \%} = \frac{\text{Loss in weight}}{\text{Weight of sample}} \times 100$$

Dry matter % = 100 - Moisture percent

3.6.2 Crude Protein (CP)

By digesting 1 g dried sample with 30 ml concentrate H_2SO_4 and adding 5 g of digestion mixture made of sodium sulphate and copper sulphate in a 9:1 ratio, the crude protein content was determined using Kjeldahl's method. The digestion was continued for another 2-3 hours till it was clear. After repeated washes with distilled water, the digested content was transferred to a 100 ml volumetric flask, and a 100 ml volume was made. A 10 ml aliquot was then fed to the distillation assembly, along with 15 ml of 40% NaOH solution. Ammonia vapours were trapped in 15 ml of Tashiro's indicator, which was made by mixing 2 percent

boric acid in 1000 ml with 200 ml pure alcohol, 12 ml methyl red (0.1%), and 6 mL bromocresol green (0.1%). A standard N/10 H_2SO_4 solution was used to titrate the ammonia boric acid complex (ammonium borate). The crude protein content was calculated using the formula mentioned below, with 1 ml of N/10 H_2SO_4 = 0.0014 g nitrogen.

$$\text{Crude protein \%} = \frac{\text{Vol. of N/10 } H_2SO_4 \text{ x 0.0014 x 6.25 x Aliquot prepared}}{\text{Aliquot taken for distillation x Wt. of sample}} \text{ x } 100$$

In 100 g of protein, 16 g of nitrogen is present on average, so 1 g of nitrogen equals 6.25 g of protein. (Factor 6.25 to convert nitrogen into protein)

3.6.3 Ether Extract (EE)

The ether extract was estimated by extracting 3 g of dried sample in Soxhlet assembly for about 7 hours with petroleum ether (60-80°C). The extraction flask was placed in a hot air oven at 100°C for 1 hour after extraction, cooled in a desiccator and weighed. The weight of fat extracted by the petroleum ether is recovered in the receiver flask, and the extract was computed using the formula below.

$$\text{Ether extract \%} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \text{ x } 100$$

3.6.4 Crude Fibre (CF)

The crude fibre was determined by adding 200 ml of 1.25 percent sulphuric acid to a 2 g moisture and fat-free sample in a spoutless beaker for 30 minutes, timed from the start of boiling and then adding 200 ml of 1.25 percent NaOH solution. After then, the contents were filtered through a muslin cloth. The crucible residue was heated in a hot air oven overnight at 80-110°C to create a consistent weight. The wastes were then burned at 600°C for 2-3 hours in a muffle furnace, cooled and weighed again. The weight lost due to ignition was the weight of crude fibre. The crude fibre was estimated using the formula below.

$$\text{Crude fibre \%} = \frac{\text{Weight of crude fibre}}{\text{Weight of sample}} \times 100$$

3.6.5 Total Ash (TA)

For total ash estimate, a 3 g dried sample was weighed in a silica crucible, decarbonized on a heater to remove smoke and then ignited at 600^0C for 2-3 hours in a muffle furnace. The crucible was retrieved from the furnace using metal tongs, cooled in a desiccator, and weighed. The weight of ash is the weight of the content in the crucible after ignition, and it was determined using the formula below.

$$\text{Total Ash \%} = \frac{\text{Weight of total ash}}{\text{Weight of sample}} \times 100$$

3.6.6 Nitrogen Free Extract (NFE)

The amount of NFE was determined on a DM basis using the formula below.

$$\text{NFE\%} = 100 - (\%\ \text{CP} + \%\ \text{EE} + \%\ \text{CF} + \%\ \text{Total ash})$$

3.7 Fibre Fraction Analysis

The fibre fractions in feed ingredients, pelleted complete feed and faecal samples obtained during digestibility were determined as per Van Soest (1967).

3.7.1 Neutral detergent fibre (NDF)

1 g sample was placed in a spoutless beaker with 100 mL neutral detergent solution (NDS) and refluxed for 1 hour to determine NDF. The contents were filtered through the Gooch Crucible by washing them in hot water under suction and then washing them in acetone. The crucible was then dried in an oven at 100°C until it reached a consistent weight. The weight of the residue in the crucible is equal to the weight of NDF, which is computed using the formula below.

$$\text{NDF \%} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

3.7.2 Acid detergent fibre (ADF)

For ADF must be determination, 1 g of material was placed in a spoutless beaker with 100 mL acid detergent solution (ADS) and allowed to reflux for 1 hour. The contents were filtered through the Gooch Crucible by washing them in hot water under suction and then washing them in acetone. The crucible was then dried in an oven at 100°C until it reached a consistent weight. The weight of the residue in the crucible is equal to the weight of ADF, which is computed using the formula below.

$$\text{ADF \%} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

3.7.3 Acid detergent lignin (ADL)

To determine ADL, 30 mL of 72 percent H_2SO_4 was added to the weighed residues of ADF by placing the crucibles in an enamel tray. To smooth the mixture and break the lumps, the crucible was agitated with a glass rod. Glass rod was allowed to remain in the crucible while replenish it with 72 percent H_2SO4 and stirring it every hour or so while the acid drains out. After three hours, the contents were filtered under a vacuum and washed again until they were acid-free. The crucibles were dried at 100°C for 8 hours before being placed in a muffle furnace at 600^0C for three hours. The weight of lignin is computed as the weight loss by ashing after treatment with 72 percent H_2SO_4 using the formula below.

$$\text{ADL\%} = \frac{\text{Loss in weight by ashing after treated with 72 \% } H_2SO_4}{\text{Weight of sample}} \times 100$$

The difference between NDF and ADF; ADF and ADL, respectively, were used to identify hemicellulose & cellulose.

3.8 *In vivo* / animal experimentation study

Based on the results of *in vitro* study for various combinations of enzyme volume and incubation period best three combinations were select for *in vivo* study and one group with untreated cotton stalk was maintained as control.

3.8.1 Selection of experimental goats

Twenty-four growing goats, ranging between four to six months of age, with similar body weights and male to female ratio were randomly allotted to four treatment groups. The average body weight of experimental growing goats ranged between 15.04 to 15.48 kg with non-significant variation.

3.8.2 Housing and Management of Experimental goats

The goats were kept in pens with adequate ventilation, flooring and tying arrangements as well as feeding and watering facilities. Throughout the experimental period, all of the experimental growing goats were maintained on standard and similar managemental practices. Before the start of the experiment, goats were dewormed and vaccinated for PPR (Raksha PPR @ 1 ml s/c). The experimental goats were weighed every fortnightly before feeding and watering during experimental period using an electronic weighing balance. The experimental goats were offered pelleted complete feed and kept under stall fed condition.

3.8.3 Dietary treatments

The goats in the control group (T_0) were fed pelleted complete feed containing 60% cotton stalk and 40% concentrate mixture. For treatment of cotton stalk in T_1, T_2 and T_3 groups enzyme solution was prepared which contained liquid enzyme concoction and water in 50:50 proportion. Whereas, in the T_1 group, 60% cotton stalk was treated with enzyme solution @ 250 ml/kg of the cotton stalk for 6 h. While in the T_2 group, 60% cotton stalk was treated with enzyme solution @ 250 ml/kg for 8 h and in the T_3 group, 60% cotton stalk was treated with enzyme solution @ 500 ml/kg for 6 h. Isonitrogenous and isocaloric

diets were used in all groups, while the roughage to concentrate ratio was maintained as 60:40.

Table 3.1 Dietary treatment groups

Groups	Experimental diet
T0	Pelleted complete feed comprised of 60% cotton stalk and 40% concentrate mixture
T_1	Pelleted complete feed comprised of 60 percent cotton stalk treated with enzyme solution @ 250ml/kg stalk for 6 h incubation and 40% concentrate mixture
T_2	Pelleted complete feed comprised of 60 percent cotton stalk treated with enzyme solution @ 250ml/kg stalk for 8 h incubation and 40% concentrate mixture
T_3	Pelleted complete feed comprised of 60 percent cotton stalk treated with enzyme solution @ 500ml/kg stalk for 6 h incubation and 40% concentrate mixture

3.8.4 Feed Preparation and Feeding

3.8.4 (a) Ration Formulation

Considering the nutrient requirements as per ICAR (2013), goats were fed on pelleted complete feed wherein the roughage to concentrate ratio was maintained as 60:40. The details of ingredients and composition of pelleted complete feed are shown in Table 3.2.

Table 3.2 Composition of Pelleted Complete Feed (%)

Group	T_0 (Control Group)	T_1 (Liquid enzymes and water @ 125:125ml/kg for 6h)	T_2 (Liquid enzymes and water @ 125:125ml/kg for 8h)	T_3 (Liquid enzymes and water @ 250:250 ml/kg for 6h)
Ingredient	Parts	Parts	Parts	Parts
Cotton Stalk	60	60	60	60
Maize	9.60	9.60	9.60	9.60
Cotton Seed Cake	7.0	7.0	7.0	7.0
Tur Chunni	4.0	4.0	4.0	4.0
Wheat Bran	4.4	4.4	4.4	4.4
GNC	13.50	13.50	13.50	13.50
Salt	0.5	0.5	0.5	0.5
Min Mixture	1	1	1	1
%Total	100	100	100	100

Spraying of enzyme solution on shredded cotton stalk with different dilution

Incubation of treated shredded cotton stalk for different duration

Sun drying of treated shredded cotton stalk

Plate 3.4 Treatment of shredded cotton stalk for pelleted complete feed

3.8.4 (b) Procurement of feed ingredients

Cotton stalk was used as a sole roughage sources in the trial, with maize, cotton seed cake, wheat bran, and tur chunni and GNC as concentrate sources in pelleted complete feed. Cotton stalks was procured from local farmers, whereas maize, cotton seed cake, GNC, wheat bran, tur chunni, mineral mixture and common salt were procured from the local market.

3.8.4 (c) Preparation of Pelleted Complete Feed

All the required feed ingredients were grounded in a hammer mill and blended in the mixer, while mineral mixture and salt were added through a medicine hopper. To soften the complete mash feed and to improve the pellet binding, it was moistened by sprinkling some water and pellets of 8 mm diameter were prepared through a pellet machine. The pellets were then air dried until they reached optimum moisture and consistency.

3.8.4 (d) Feeding of experimental goats

Individual feeding of goat was followed during the experiment. The experimental fed was provided for 120 days according to their dietary treatments. Changes in body weights and growth rates of growing goats were considered to modify the quantity of feed to be offered. A measured quantity of pelleted feed was offered twice daily, in the morning and evening hours, and free access to fresh and clean drinking water was provided. If there was any leftover feed, it was recorded at 24 hours interval on the next day morning to compute the daily feed intake.

3.9 Body weights and body weight gain

The body weights of individual animal in each group were recorded before feeding at fortnightly interval using digital electronic weighing balance. The body weight gain was calculated fortnightly accordingly. Further from the observations of body weight gain, average daily gain (ADG) in grams were calculated and expressed fortnightly.

3.10 Dry matter intake

The record of daily feed intake was maintained for each animal and daily dry matter intake was calculated considering the dry matter % of feed. The daily dry matter intake was expressed in gram on fortnightly basis. Further DMI percent body weight was calculated from the observations of body weights for the corresponding fortnight and dry matter intake was expressed in kg.

3.11 Feed Conversion Efficiency

The feed conversion efficiency was computed as ratio of dry matter consumed (g) and body weight gain (g) on fortnightly basis.

3.12 Rumen liquor profile

3.12.1 Collection of rumen liquor

Rumen liquor samples were collected from each goat in each group using a stomach tube at monthly intervals, 2 hours post feeding. The rumen fluid was delivered to the lab in an insulated thermos and strained through a double-layer muslin cloth before being tested for pH, ammonia nitrogen, total volatile fatty acids (TVFA), and individual volatile fatty acids, total nitrogen, TCA-ppt nitrogen and NPN concentrations.

3.12.2 pH of rumen liquor

The pH of strained rumen liquor (SRL) was evaluated immediately after collection using a pen-type pH metre (Eutech model ECOTestrpH2).

3.12.3 Ammonia nitrogen

On the same day of collection, the ammonia nitrogen in rumen liquor was determined using Conway's micro-diffusion method (1957).

0.5 ml SRL was poured into the outside cell of the Conway diffusion dish, and a 1 ml boric acid indicator was inserted in the inner central chamber of the Conway diffusion dish to estimate rumen ammonia nitrogen. In the outer cell, 0.5

Feed Technology Unit of Department of Animal Nutrition, NVC, Nagpur

Grinding of feed ingredients

Pelleting of complete feed

Experimental pelleted complete feed

Plate 3.5 Preparation of pelleted complete feed

Feeding experimental goat with complete pelleted feed

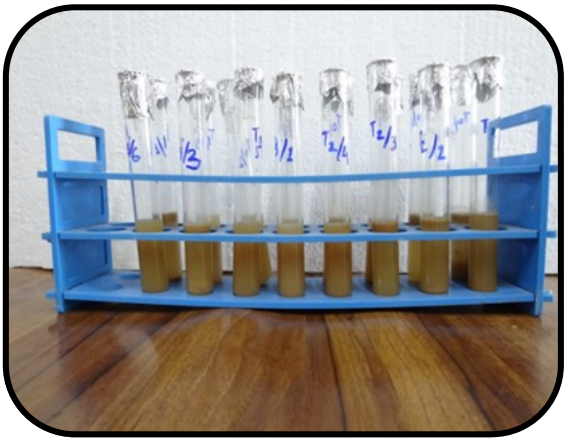
Plate 3.6 Feeding of experimental goats

Feeding experimental goat with complete pelleted feed

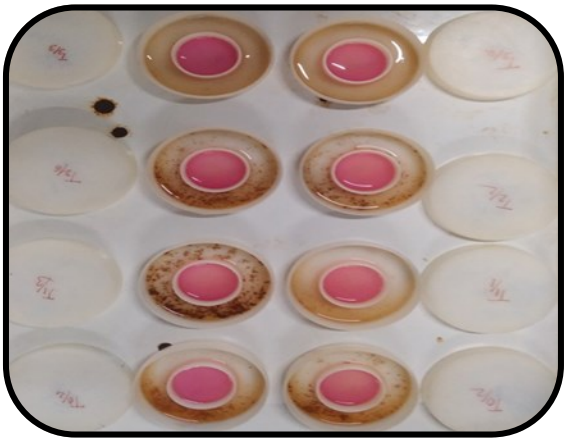
Plate 3.6 Feeding of experimental goats

Rumen liquor collection of experimental goats through Ryles stomach tube

Recording pH of freshly collected rumen liquor



Rumen liquor for TCA-ppt-N



NH_3-N by Conway diffusion dish method

Plate 3.7 Collection and laboratory analysis of rumen liquor samples

mL of 45 percent potassium carbonate solution was put opposite the SRL. The contents of the outer chamber of the Conway diffusion dish were mixed (the potassium carbonate solution with SRL) by gentle rotating/tilting the dish on a flat surface, and the dish was then kept in the incubator at 38^0C for 1-2 hours. The inner chamber's contents were then titrated with 0.001 N sulphuric acid. Total rumen ammonia nitrogen was then calculated by following formula-

Rumen NH_3-N (mg/100 mL) = mL of 0.001 N H_2SO4 used x 0.000014 x 100 x1000

3.12.4 Total volatile fatty acids (TVFA)

The total volatile fatty acids (TVFA) in rumen liquor were measured using Barnett and Reid's method (1957).

Markham's equipment was linked to a round bottom flask (2 lit.) half filled with water, kept on the heating mantle, and allowed the water to boil for TVFA estimate in SRL. In the Markham's steam distillation apparatus, 2 mL SRL and 2 mL oxalate buffer (10 percent potassium oxalate + 5 percent oxalic acid) were pipetted and left to boil. About 100 mL of distillate was collected in an ice-cold conical flask, which was then titrated with 0.01N sodium hydroxide solution and 2 drops of phenolphthalein indicator until a pink colour developed. The TVFA was then determined using the formula below.

$$\text{TVFA mEq/L SRL} = \frac{\text{Volume of NaOH used x strength of NaOH}}{\text{Volume of rumen liquor taken}} \times 1000$$

3.12.5 Total nitrogen

On the same day of collection, the total nitrogen in rumen liquor was determined using Kjeldhal's method (AOAC, 2005).

5 ml SRL, 10 mL conc. H_2SO_4, and 2-3 g digestion mixture (K_2SO_4 + $CuSO_4$ in a 9:1 ratio) were combined in a Kjeldhal flask and digested for 2 hours at 70° C until the solution turned transparent blue. After cooling, it was transferred to a 100 ml volumetric flask. After that, a 100 mL aliquot was made by diluting the digested material with distilled water. The released ammonia was collected in

a 2 percent boric acid reagent in the conical flask and titrated against 0.01 N sulphuric acid solution after distillation on the micro Kjeldhal distillation assembly with 10 mL of 40 percent NaOH. using burette. After titration total nitrogen was then calculated by following formula-

$$\text{Total nitrogen mg/dl SRL} = \frac{\text{0.01 N } H_2SO_4 \text{ sol. used x 0.00014 x Aliquot prepared x 100}}{\text{mL of SRL x aliquot taken}} \text{ x } 1000$$

3.12.6 TCA-precepitable nitrogen

On the same day of collection, the TCA-ppt in rumen liquor was calculated using Kjeldhal's technique (AOAC, 2005). 5 mL SRL was placed in a centrifuge tube with 5 mL 20 percent tri-chloro acetic acid solution and maintained overnight for TCA-ppt-N determination. It was then centrifuged for 10 minutes at 2,000 rpm. Following centrifugation, the entire precipitate was rinsed with distilled water in a Kjeldhal flask before being treated for digestion, distillation, and titration as described above for total nitrogen measurement.

$$\text{TCA-ppt-N mg/dl} = \frac{\text{0.01 N H2SO4 sol. used x 0.00014 x Aliquot prepared x 100}}{\text{mL of SRL x aliquot taken}} \text{ x } 1000$$

3.12.7 Non protein nitrogen

By deducting TCA-ppt nitrogen from the total nitrogen content of SRL, the NPN content of rumen liquor was estimated.

3.13 Digestibility trial

To determine the digestibility of nutrients, a digestibility trial with 5 day collection period was conducted on experimental goats during last week of experiment. During this period, arrangements were established for quantitative faecal collection. During the trial time, a detailed record of the feed consumed, residual left, and excrement voided by each experimental goat was kept. After every 24 hours, the amount of excrement voided by each goat was collected and weighed quantitatively at 8:00 a.m., and a uniform representative sample of the residue was retained in a labelled polythene bag. The residual feed from each goat

Digestibility trail for experimental goats

Collection of faecal sample during digestibility trail

Plate 3.8 Digestibility trail

Analysis of blood serum samples

Straining of rumen liquor through four-layer muslin cloth

Plate 3.9 Laboratory analysis

after 24 hours of consumption was weighed to calculate the daily feed intake. The representative samples of feed and faeces collected throughout the digestibility trial were analyzed for the proximate composition according to AOAC (2005) and fibre fraction according to Van Soest (1967).

3.14 Blood biochemical study

Blood samples were collected at zero days and then monthly intervals of each and every animal from jugular vein into sterilized syringes during the morning before the feeding of experimental growing goats of each group in clot activator tubes. The serum was separated and stored in deep freeze for further analysis. Blood biochemical parameters were analyzed using commercial kits on an auto analyzer (Microlab-300). Total serum protein was determined as per the procedure laid down by Doumas (1975) and serum albumin as per Doumas and Watson (1971) and globulin by subtraction. The blood urea nitrogen was estimated as per Chaey (1962).

3.15 Economics of feeding

The economics of feeding was calculated considering the prevailing prices of feed and fodder materials, as well as ancillary costs and the body weight gain and ultimately, cost of feeding per unit of body weight gain.

3.16 Experimental design and Statistical Analysis

The data collected during the study were analyzed applying Three-way Factorial and Completely Randomized Design as per procedure prescribed by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

4.1. Extraction, purification and assay of enzymes

The study with regard to extraction and purification of enzymes, quantitative analysis (assay) of enzymes, degradation degree and thermal stability of enzyme was made. The observations and results obtained during present study have been interpreted

4.1.1 Extraction and purification

Enzyme extraction and purification study was carried out in the Department of Chemical Engineering, Visvesvaraya National Institute of Technology, Nagpur. The shredded rice straw (1 x 2 mm) was diluted to biomass: water: Bajrang Ban (a patented consortia of microorganisms developed indigenously at VNIT) (Mandavgane and Chakravarty, 2021) in 1:1:0.5 in a container and set for fermentation for 14-15 days (residence time) in nearly anaerobic condition. Phosphate buffer (Na_2HPO_4 and NaH_2PO_4) of pH 6 was used for extraction and the contents were soaked overnight with maintaining solid to liquid ratio at 1:10. Then it was centrifuged at 10000 rpm for 15 min at $<10^0$C for lysis of remaining microbial cells in buffer extract. Four litre extract per cycle was separated by membrane separator MWCO - 75k Da, operated under 2 bar pressure to prevent damage to membrane. Filtration rate was 0.756 L/min and column life up to 6 kL.

4.1.2 Quantitative analysis

The cellulase activity was estimated using filter paper assay. Enzyme standard was procured from SRL Chemicals derived from *Trichoderma viride* with activity 10000 U/g. Enzyme stock solution was prepared by 10 mg of mixing standard enzyme in 10 mL of buffer solution. The activity of the stock was calculated to be 10 U/mL. Enzyme stock was diluted into five different standard samples, with dilution rates of 0.03, 0.04, 0.05, 0.08 and 0.09. Glucose standard curve was plotted with sample concentrations of 2.00, 3.30, 5.00 and 6.70 mg/mL

using dextrose. From the standard curve, corresponding reducing sugar concentrations were calculated to be 0.095 mg/mL. It was observed that the total cellulase enzyme activity was found to be 373.5 U/L.

The xylanase assay was made by DNS method (Miller,1959) and was based on the production of reducing sugar from a solution of xylan and the reducing sugar was then reacted with 3,5 Dinitrosalicyclic Acid (DNS). The said assay was done at Cargill Premix & Nutrition – India and was reported to be 1159.45 U/L.

Use of Solid Substrate Fermentation (SSF) for enzyme production has been studied extensively by Delabona *et. al.* (2012) who also reported activities of three types of cellulase enzymes, up to 160.1 IU/g for CMCase (endo-glucanase), 5.0 FPU/g for FPase (exo-glucanase), 105.82 IU/g for β-glucosidase and 1055.62 IU/g for xylanase by using Amazonian wood fungi over common agricultural residues like sugarcane bagasse, orange peels and wheat brans.

Production of enzymes and their respective activities is dependent on several factors- nature of substrate, nature of microorganisms, moisture and temperature conditions, to name a few. The activity profile of the enzyme cocktail produced in this experiment shows greater xylanase activity over cellulase, as reported by Delabona *et. al.* (2012). Cellulase activities in the range of 5.69 U/g to 106 U/g have been reported by Salihu *et. al.* (2015), Kholif *et. al.* (2017)[a], Ijoma *et. al.* (2018) and Astolfi *et. al*. (2019).

4.1.3 Degradation degree

The degradation experiments were carried out for a period of six days. 5 g of biomass (rice straw) was mixed with 40 mL of dilute enzyme solution in six identical flasks. Whole flask cultures were considered for evaluation of degradation efficiency. Flasks were opened per day to depict the degradation pattern for each day. Control flask was added with 40 ml of distilled water instead of enzyme solution. It was observed that the release of sugar flat lined after day 4, indicating a cessation in enzymatic action over cellulosic and hemicellulosic

fibres. The present study, could correlate the increased bioavailability of reducing sugars with increase in unfolding of complex lignocellulosic structures due to enzyme action, with overall fraction reaching up-to 94% increase in availability in a matter of 3-4 days.

Similar findings have been reported by Yoshida *et. al.* (2008) and Choi *et. al.* (2010) and reported increased availability of reducing sugars as a result of degradation of complex lignocellulosic structures due to enzyme action.

4.1.4 Thermal stability

For estimation of thermal stability, the dilute enzyme was subjected to vacuum drying for 6 hours at three different temperatures- 40, 50 and 60 °C. As an indicator for thermal stability, the active status of enzyme solution was estimated by performing degradation studies on rice straw. 5 g of sample (rice straw) was treated with 20 mL of enzyme and 20 mL of water and incubated for 4 days. The biomass obtained after the end of incubation was dried and estimated for cellulose and hemicellulose. It was observed that the enzyme remained stable even at temperatures as high as 60°C. It was also observed that the degradation efficiency, owing to the reduced solvent content, thus concentrated the enzyme.

Present observations are in agreement with Delabona *et al.* (2012) who reported that the enzymes produced in the process tend to be thermostable, up to 75°C, although maximum activity was observed in case of incubation temperature of 65°C. Further, Astolfi *et al.* (2019) reported enzyme stability at 40°C at an acidic range of pH (4.5-5.5) used soybean husk as substrate as agro-residues for the production of enzymes for application in biofuel production with *Trichoderma reesi* was inoculated for solid substrate fermentation which supported the present findings.

4.2 Standardization of dose and duration of treatment of fiber degrading enzymes in the shredded cotton stalk

After preparation and quantification of liquid fibrolytic enzymes, standardization of doses of liquid fibrolytic enzymes treatment was carried out.

For this, initially shredded cotton stalk was soaked with enzymes solution (50% liquid enzymes preparation and 50% water) at the dose rate of 8 L/kg of shredded cotton stalk (1:8) for four identical batches and incubated for 24, 48, 72 and 96 h. After incubation, large volume of leachate (a solution resulting from leaching) remained in the soaked shredded cotton stalk measuring 4.1, 4.0, 3.9 and 3.8 L in 24, 48, 72 and 96 h incubated samples respectively. Further, the obtained leachate was subjected for analysis of reducing sugar by Brick Meter which was found to be 2.5, 2.5, 2.7 and 2.8% in leachate soaked for 24, 48, 72 and 96 h respectively. It is indicated that more reducing sugars escaped in leachate when dilution (dose) and Incubation period was increased. The observation thus indicated that the volume of enzyme solution needs to be reduced for the treatment of cotton stalk.

In view of above findings, the dose of enzymes solution was reduced from 1:8 to 1:4 ratio in second trial. For this shredded cotton stalk was soaked with enzymes solution at the dose rate of 4 L enzyme solution /kg cotton stalk (1:4) for four identical batches and incubated for 24, 48, 72 and 96 h. After incubation, volume of leachate obtained in soaked shredded cotton stalk was measured to be 1.8, 1.6, 1.5 and 1.3 L in 24, 48, 72 and 96 h incubated samples, respectively. Further, the reducing sugar was found to be 2.5, 2.5, 2.6 and 2.6% in leachate obtained in 24, 48, 72 and 96 h incubated samples respectively. It is concluded that though reducing sugar was less in 1:4 as compare to 1:8 dilutions, the notable amount of leachate was obtained. The treated cotton stalk samples @ 1:4 ratio (incubation for 24, 48, 72 and 96 h) were also subjected for assessing the *in vitro* dry matter digestibility.

Table 4.1 *In vitro* DM digestibility (%) of cotton stalk treated with enzyme solution at 1:4 ratio

Treatment	Incubation period (h)	*In-vitro* dry matter digestibility (%)
Enzyme solution 4 L/kg of cotton stalk (1:4) (w/v)	24	$48.44^{d}\pm0.64$
	48	$43.35^{c}\pm0.69$
	72	$34.65^{b}\pm1.50$
	96	$30.17^{a}\pm2.20$

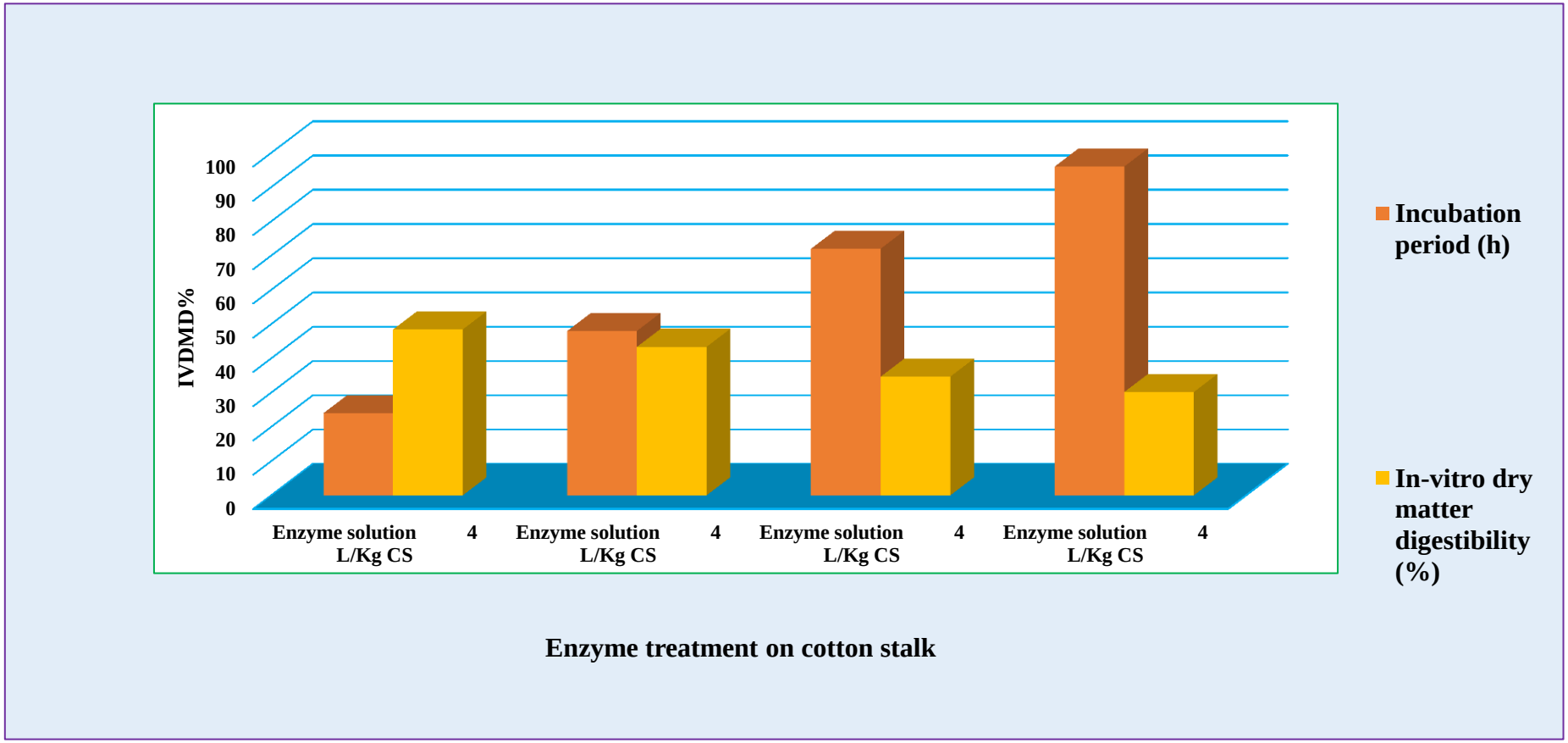


Figure 4.1 *In vitro* DM digestibility (%) of cotton stalk treated with enzyme solution at 1:4 ratio

The observations of *in-vitro* dry matter digestibility (%) of cotton stalk treated with enzymes solution with 1:4 (w/v) ratio and for 24, 48, 72 and 96 h incubation duration are presented in Table 4.1 and depicted graphically in Fig. 4.1. In this study, the dry matter digestibility (%) was found to be 48.44±0.64, 43.35±0.69, 34.65±1.50 and 30.17±2.20 in enzyme solution treated shredded cotton stalk samples soaked for 24, 48, 72 and 96 h, respectively with significant variation ($p<0.05$). The observations revealed decrease in *in vitro* dry matter digestibility with the increase in incubation period that could be attributed to the action of fibrolytic enzyme on cellulose content in cotton stalk and its breakdown into simple sugars *i.e.* glucose which escaped in the leachate.

In view of above outcome, the dose of enzyme treatment was further reduced and it was concluded to conduct *in vitro* digestibility determination trial on shredded cotton stalk sprayed with enzymes solution @ 250 ml/kg (1:0.250) and 500 ml/kg (1:0.500) and allowed incubation for 4, 6 and 8 h duration.

4.3 *In vitro* digestibility determination study of enzyme treated cotton stalk

The detailed *in vitro* digestibility determination study with regard to cotton stalk sprayed with enzymes solution @ 250 ml/kg (1:0.250) and 500 ml/kg (1:0.500) and soaked for 4, 6 and 8 h duration was carried out in this experiment. It was decided to select best three treatments from the results obtained in the *in vitro* study for further validation with *in vivo* studies on goats.

4.3.1 Composition of untreated and enzymes treated cotton stalk

The samples of cotton stalk and liquid enzymes treated cotton stalk were analyzed for its proximate and fiber composition. The results obtained have been presented in Table 4.2.

Table 4.2 Proximate and fiber composition of enzyme treated and untreated cotton stalk (% DM basis)

Nutrients (%)	Untreated cotton stalk	Enzymes treated cotton stalk					
		Enzyme solution @ 250 ml/kg of CS			Enzyme solution @ 500 ml/kg of CS		
		Incubation period (h)					
		4	6	8	4	6	8
Dry matter	92.46	91.45	90.19	91.25	91.01	90.58	91.45
Organic matter	89.44	89.88	88.42	88.75	89.44	88.42	89.74
Crude protein	3.12	3.22	3.01	3.10	3.11	3.25	3.31
Ether extract	1.06	1.05	1.06	1.11	1.02	1.30	1.12
Crude fiber	52.46	48.26	46.56	49.15	47.55	45.10	49.56
NFE	32.80	37.35	37.79	35.39	37.76	38.77	35.75
Total ash	10.56	10.12	11.58	11.25	10.56	11.58	10.26
NDF	81.45	76.57	73.20	76.28	77.58	72.29	77.15
ADF	69.54	62.54	59.32	60.54	62.52	58.28	61.58
ADL	51.68	45.18	39.65	39.67	44.55	38.28	39.15
Hemicellulose	11.91	14.03	13.88	15.74	15.06	14.01	15.57
Cellulose	17.86	17.36	19.67	20.87	17.97	20.00	22.43

The composition and fiber fraction study revealed greater percent CF in untreated cotton stalk than the enzyme treatment groups while, percent NFE was found to be lesser in untreated cotton stalk than the treated groups. The NDF, ADF and ADL composition of untreated cotton stalk was observed to be higher than cotton stalk given enzyme treatment groups with combinations of dose and incubation duration. However, hemicelluloses and cellulose content in various treated groups was higher with exception of cellulose content in group treated with 250 ml/kg and 500 ml/kg cotton stalk for 4 h wherein it was nearly similar to untreated cotton stalk. The observations recorded in present study are in accordance with Jadhav (2019) and Burghate (2021), who reported analogous finding with regard to proximate and fiber composition of untreated cotton stalk. However, study of nutrient composition and fiber fraction of cotton stalk made by Grewel *et al.* (2003), Nagalakshmi and Reddy (2011) and Hashim *et al.* (2017) deviate from the present findings, which may be due to variations in chemical

composition, variety of cotton crop, thrashing methods, maturity of plant at the time of harvesting, soil and other factors affecting plant composition.

4.3.2 *In vitro* studies

The *in vitro* digestibility of cotton stalk treated with enzymes solution @ 250 ml/kg (E_{250}) and 500 ml/kg (E_{500}) and incubated for 4, 6 and 8 h duration was carried out. Similar volume and incubation period was also taken for water treatment so as to observe the distinctive action of liquid enzyme preparation over the effect of water treatment (W_{250} and W_{500}) of cotton stalk. The results obtained are presented in Table 4.3 and 4.4 and depicted graphically in Fig.4.2 and 4.3.

Table 4.3 *In vitro* DM digestibility (%) of cotton stalk subjected to different treatment combinations

Particulars	Incubation period (h)	Enzyme Solution @ 250 ml/kg of CS (E_{250})	Enzyme Solution @ 500 ml/kg of CS (E_{500})	Water @250ml/kg of CS (W_{250})	Water @500ml/kg of CS (W_{500})
		Enzyme Treatment		Water Treatment	
IVDMD (%)	4	58.14^{b}±1.31	55.36^{a}±0.67	56.02^{a}±0.69	58.12^{b}±0.92
	6	66.04^{b}±0.88	67.18^{c}±2.05	54.02^{a}±0.87	56.10^{a}±0.71
	8	63.62^{c}±0.92	57.66^{b}±0.91	53.22^{a}±0.71	58.28^{b}±0.78
	Pooled Mean	62.60±1.91	60.07±2.95	54.42±0.68	57.50±0.57

^{abc} Means with different superscripts within rows and columns differ significantly ($p<0.01$), CD-3.74

The average *in vitro* dry matter digestibility % of representative sample of untreated cotton stalk was assessed and found to be 42.10±0.41. The *in vitro* digestibility for DM of cotton stalk treated with liquid fibrolytic enzyme solution for the different treatment groups revealed significantly higher ($p<0.01$) *in vitro* DM digestibility in E_{250} group for 6 & 8 h and E_{500} group for 6 h with 66.04±0.88, 63.62±0.92 and 67.18±0.05 percent *in vitro* DM digestibility, respectively than other groups with enzyme and water treatment. The IVDMD values in enzyme treated groups were higher than its corresponding water treatment groups, exhibiting distinct and beneficial effect of treatment with enzyme solution on the dry matter digestibility of cotton stalk.

In vitro digestibility of DM in E_{250} group (63.62%) for 6 and 8 h as well as in W_{500} group for 6 h incubation is in agreement with Lunagariya *et al.* (2017), who reported higher IVDMD with higher level of incorporation of exogenous fibrolytic enzymes. Similarly, Sheikh *et al.* (2017) reported significant improvement *in vitro* digestibility of DM due to probiotics and fibrolytic enzyme supplementation in paddy straw.

Table 4.4 *In vitro* NDF and ADF digestibility (%) of cotton stalk subjected to different treatment combinations

Particulars	Incubation period (h)	Enzyme Solution @ 250 ml/kg of CS (E_{250})	Enzyme Solution @ 500 ml/kg of CS (E_{500})	Water @250ml/kg of CS (W_{250})	Water @500ml/kg of CS (W_{500})
		Enzyme Treatment		Water Treatment	
IVNDFD (%)	4	70.45^{c} ±2.31	73.15^{c} ±1.67	64.08^{b} ±0.66	63.56^{b} ±0.42
	6	78.74^{de} ±1.04	80.46^{e} ±1.90	57.02^{a} ±1.00	61.80^{ab} ±0.46
	8	72.51^{c} ±2.58	74.80^{cd} ±1.65	64.46^{b} ±0.90	61.71^{ab} ±0.83
	Pooled Mean	75.63±1.80	77.63±1.63	60.74±2.15	61.76±0.03
IVADFD (%)	4	58.43^{abc} ±1.89	60.16^{bcd} ±1.70	57.16^{ab} ±0.41	62.14^{cde} ±0.49
	6	65.81^{e} ±1.04	66.35^{e} ±1.79	53.76^{a} ±0.91	57.30^{ab} ±0.56
	8	64.76^{de} ±1.52	58.81^{bc} ±0.75	62.80^{cde} ±0.57	60.24^{bcd} ±1.07
	Pooled Mean	65.29±0.30	62.58±2.18	58.28±2.61	58.77±0.85

^{a,b,c,d,e} Means with different superscripts within rows and columns differ significantly ($p<0.01$), CD for NDF-5.13 and CD for ADF 4.79

The significantly higher ($p<0.01$) IVNDFD (%) was evident in (E_{500}) group for 6 h (80.46±1.90) and in (E_{250}) group for 6 h (78.74±1.04) and 8 h (74.80±1.65) in (E_{500}) group as compared to other groups. Enzyme treatment groups also showed better IVNDFD values than its corresponding water treated groups. Rajamma *et al.* (2015) also reported significantly higher IVNDFD (%) in exogenous fibrolytic enzymes (EFE) supplemented groups in TMR-1 (60R:40C) and TMR-2 (70R:30C) than without EFE supplementation which is in tune with present findings. Similarly, Reddy *et al.* (2016) reported that *in vitro* digestibility (%) of NDF increased linearly in treatment group supplemented with EFE and/or

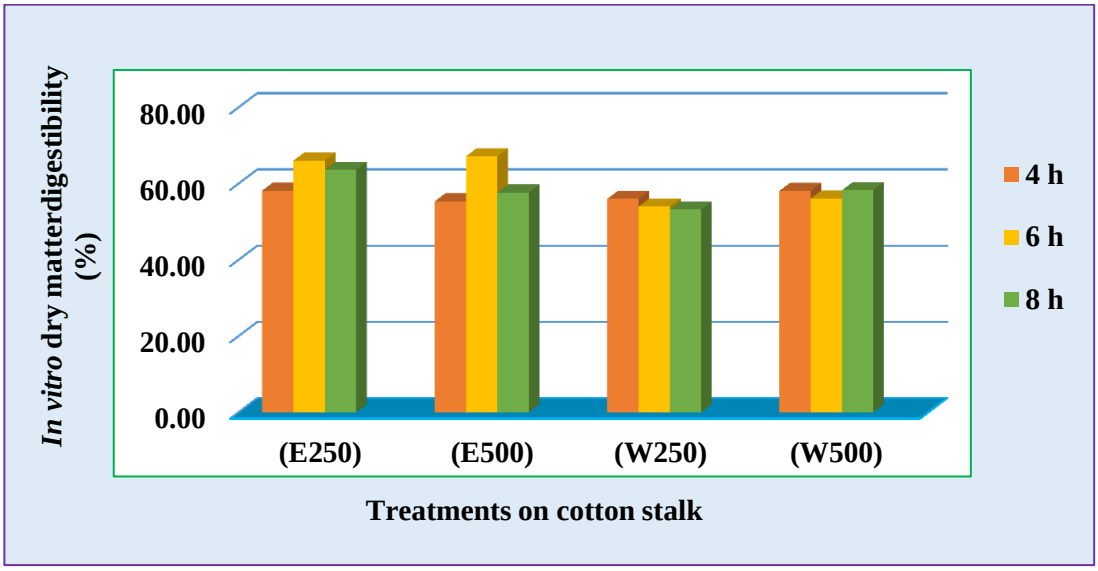


Figure 4.2 *In vitro* DM digestibility (%) of different treatment combinations on cotton stalk

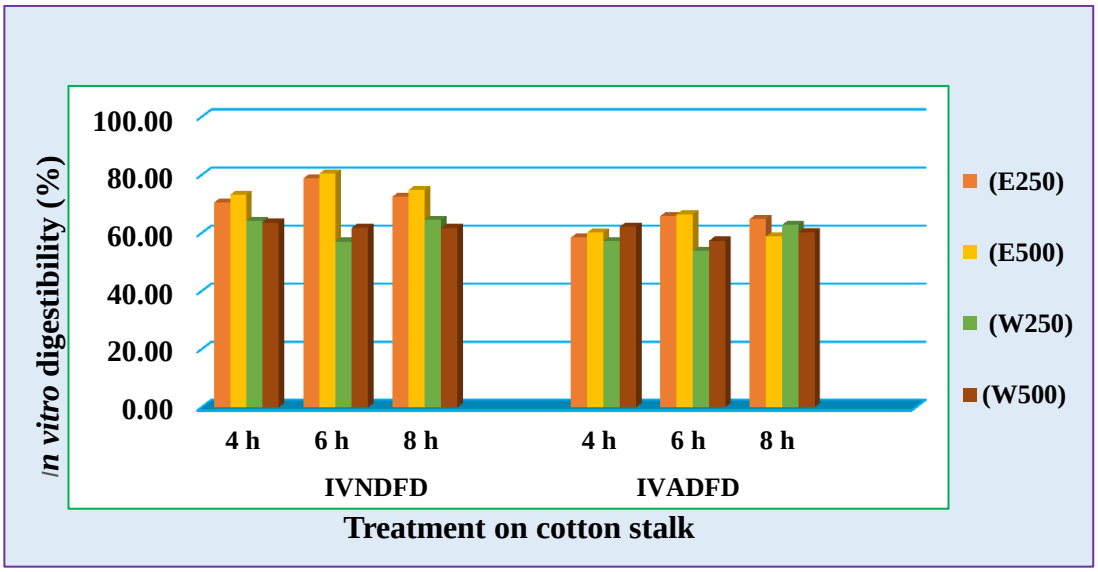


Figure 4.3 In vitro NDF and ADF digestibility (%) of cotton stalk subjected to different treatment combinations

live yeast culture in TMR (70R:30C) than control TMR (70R:30C) group that are comparable with observations in present study.

In vitro acid detergent fiber digestibility (%) was significantly higher ($p<0.01$) in (E_{250}) group for 6 and 8 h (65.81±1.04, 64.76±1.52) and in (E_{500}) group for 6 h (66.35±1.79). Cotton stalk treated with enzyme solution might have caused fiber hydrolysis and/or solubilization of ADF which might have increased digestion rate. Thus, the enzyme treatment was effective to improve IVADFD in cotton stalk over the corresponding water treatment groups, indicating beneficial effect of enzyme treatment of cotton stalk. Also, the present findings can be seen on the background that enzyme application to the substrate enhances the attachment of rumen microorganism to the feed particles, thereby increasing the hydrolytic capacity (Wang *et al.,* 2001).

From the above observations made in present study with regard to *in vitro* digestibility, it can be concluded that IVDMD, IVNDFD and IVADFD increased due to liquid fibrolytic enzyme treatment of cotton stalk, however enzyme solution treatment of cotton stalk @ 500 ml/kg incubated for 6 h proved to be superior followed by enzyme solution treatment @ 250 ml/kg incubated for 6 and 8 h. Therefore, these three best treatment combinations were selected for further *in vivo* experimentation on goats.

4.4 *In vivo* studies/animal experimentation on performance of goats

Based on the findings of *in vitro* digestibility studies made during standardization of enzyme treatment of cotton stalk, the best three combinations of dose of enzyme solution and incubation time were used for treatment of cotton stalk and for its further use as roughage source in pelleted complete feed for experimental goats. For comparison, group maintained on pelleted feed without enzyme treatment of cotton stalk was kept as control. For *in vivo* studies, a feeding trial was conducted for 120 days duration on 24 non-descript growing goats randomly allotted to 4 experimental dietary treatment groups (T_0, T_1, T_2 and T_3) to observe the effect of different dietary treatments on various parameters such as body weight gain, dry matter intake, feed conversion ratio, nutrient

digestibility, rumen liquor parameters, blood biochemicals and economics of feeding. The findings and results obtained during *in vivo* study have been presented and discussed below-

4.4.1 Chemical Composition of feed ingredients and experimental diet (% DM basis)

The cotton stalk procured from farmer's field and different concentrate feed ingredients procured from market were analyzed for its proximate analysis and fiber fraction composition. The results of the analysis are presented in Table 4.5.

Table 4.5 Chemical composition of feed ingredients used for *in vivo* study (% DM basis)

Particulars (%)	Ingredients					
	Maize	CSC	GNC	Arhar chunni	Wheat bran	Untreated cotton stalk
Dry matter	91.60	90.46	90.02	91.66	90.66	92.46
Organic matter	98.78	94.54	94.20	95.44	93.46	89.44
CP	9.22	27.72	43.01	16.54	14.56	3.12
EE	3.18	8.64	1.40	4.02	3.02	1.06
CF	6.44	12.86	5.70	16.72	10.12	52.46
NFE	79.94	45.32	46.89	56.16	65.76	32.80
TA	1.22	5.46	3.00	6.56	6.54	10.56
NDF	26.44	32.40	16.70	29.66	45.22	81.45
ADF	19.20	23.12	9.30	17.12	26.72	69.54
ADL	2.24	8.46	1.20	1.26	9.98	51.68
Hemicellulose	7.24	9.28	7.40	12.54	18.50	11.91
Cellulose	16.96	14.66	8.10	15.86	16.74	17.86

The complete diets for dietary treatment groups were formulated as per ICAR (2013) so as to meet the dietary requirements of experimental goats. The experimental goats were offered pelleted complete feed prepared as per plan of *in vivo* experiment. The chemical composition of experimental diets (pelleted complete feed) was carried out and is presented in Table 4.6.

Table 4.6 Chemical composition of complete feed pellets (%DM basis)

Nutrients (%)	Treatments			
	T_0	T_1	T_2	T_3
Dry matter	91.00	91.12	89.72	90.98
Organic matter	91.74	91.33	91.52	91.32
Crude protein	11.64	11.59	11.64	11.73
Ether extract	1.95	1.97	1.98	2.09
Crude fibre	32.74	31.20	32.75	30.32
Nitrogen free extract	45.41	46.57	45.15	47.18
Total ash	8.26	8.67	8.48	8.68
NDF	58.94	53.99	55.84	53.45
ADF	48.19	42.06	42.79	41.43
ADL	32.43	25.20	25.22	24.39
Hemicellulose	10.75	11.93	13.05	12.02
Cellulose	15.76	16.86	17.57	17.04
DCP (Calculated)	9.54	9.54	9.54	9.54
TDN (Calculated)	58.41	58.41	58.41	58.41

The proximate composition and fiber fraction of the pelleted complete feeds indicated all experimental groups received similar dietary nutrient composition through feed and that the complete feed pellets offered to all experimental groups had similar DCP and TDN content and depicts iso-nitrogenous and iso-caloric diet.

4.4.2 Average fortnightly body weights of goats

The present experiment on treatment of cotton stalk by different dilution of fibrolytic enzymes was attempted to explore the possibility of utilization of cotton stalk fully as roughage source in pelleted complete feed for growing goats. Therefore, body weights of goats in all experimental groups were recorded at fortnightly interval. The fortnightly body weights in goats for different treatment groups are presented in Table 4.7 and depicted graphically in Fig. 4.4.

Table 4.7 Average fortnightly body weight in experimental goats (kg)

Fortnights	Groups				Significance
	T_0	T_1	T_2	T_3	
0th day	15.04^{a}±1.79	15.41^{a}±1.78	15.49^{a}±1.45	15.22^{a}±2.54	NS
15th day	15.85^{ab}±1.81	16.30^{ab}±1.87	16.39^{ab}±1.48	16.25^{a}±2.57	NS
30th day	16.81^{abc}±1.84	17.28^{abc}±1.96	17.40^{abc}±1.51	17.47^{ab}±2.69	NS
45th day	17.89^{abc}+1.86	18.28^{abc}±2.04	18.51^{abc}±1.54	18.68^{abc}±2.75	NS
60th day	18.97^{bcd}±1.89	19.39^{bcd}±2.12	19.62^{bcd}±1.55	19.86^{bcd}±2.87	NS
75th day	20.17^{cde}±1.92	20.59^{cde}±2.19	20.74^{cde}±1.54	21.23^{cde}±2.98	NS
90th day	21.36^{de}±1.98	21.65^{cde}±2.26	21.90^{cde}±1.56	22.35^{de}±3.09	NS
105th day	22.62^{e}±2.01	22.80^{de}±2.32	23.11^{de}±1.57	23.61^{e}±3.11	NS
120th day	23.65^{e}±2.04	23.94^{e}±2.40	24.18^{e}±1.58	24.79^{e}±3.18	NS
Pooled Mean	19.15±0.70	19.52±0.75	19.70±0.61	19.94±0.98	

$^{a,b,c.d.e}$ –Means bearing different superscript in a column differ significantly (p<0.01), CD- 3.58
NS – Non significant

The body weights on 0th day in all groups did not differ significantly and were comparable. The initial average body weights in experimental goats were recorded to be 15.04±1.79, 15.41±1.78, 15.49±1.45 and 15.22±2.54 kg for T_0, T_1, T_2, and T_3 groups, respectively. The non-significant differences amongst the body weights for different groups indicated homogenous distribution of animals for the experiment. Similarly, the body weights recorded at different fortnightly interval revealed non- significant differences between the treatment groups. The corresponding body weights at the end of experiment *i.e.* on 120th day were observed to be 23.65±2.04, 23.94±2.40, 24.18±1.58 and 24.79±3.18 kg, respectively. The corresponding body weights at the end of experiment though exhibited higher body weights in treatment groups as compared to control (To) group, however the difference was not statistically significant. The pooled mean of body weights for the treatment groups were 19.15±0.70, 19.52±0.75, 19.70±0.61 and 19.94±0.98 kg for T_0, T_1, T_2 and T_3 group, respectively and even there was no significant difference in body weight among control and treatment groups but, numerical increase in treatment groups was observed over the control group. The higher body weights were observed in T_3 group followed by T_2, T_1 and T_0 groups in decreasing order.

Each group exhibited physiological increase in body weights with the advancing age, however, the body weights in each group were comparable at respective fortnights. The results thus revealed that, there is no adverse effect on body weight of growing goats due to pre-treatment of fibrolytic enzymes like cellulase and xylanase on cotton stalk rather marginally supported the growth performance of goats even on high fibrous and lignified cotton stalk as roughage source in pelleted complete feed as compared to control group.

The treatment of cotton stalk with combination of enzymes was hypothesized to be beneficial for growing goats via different actions, mainly by improving fiber digestion due to the action of specific fibrolytic enzymes, and by maintaining rumen eubiosis and microbial activity due to the action of enzymes as well as the positive effects on health and metabolism. However, in the present study pelleted complete feed containing enzyme treated cotton stalk showed numerical benefit in terms of body weight over the control group. It could be due to positive effect of enzyme treatment of cotton stalk on carbohydrate degradation of cell walls (Krueger and Adesogan, 2008; Tang *et al.*, 2008), co-operating in the feed hydrolysis, its synergistic interaction with rumen micro-organism to enhance the digestion of feed ingredients (Beauchemin *et al.* 2003). However, using high lignocellulytic ingredients particularly agricultural residues, the results could be variable and inconsistent in terms of fiber digestibility because potential of enzymes depends upon the dose and substrate, but in the present study enzymes acted favorably to cause marginal enhancement in the body weights of goats receiving enzyme treated feed.

The findings in the present study are in agreement with Salem *et al.* (2011) and Torres *et al.* (2013) who stated that there was significantly increase in body weights as the age advances; however, the body weights were comparable between untreated and treatment group supplemented with cocktail of enzymes and different doses of enzymes, respectively.

Kewan *et al.* (2019) reported comparable value of final live weight between fungi and yeast treatment and attributed due to improved quality of

Moringa stalks treated with fungus and yeast which probably enhanced the utilization and the availability of essential nutrients.

4.4.3 Average fortnightly body weight gain

The record of body weight determined at fortnightly interval were used to calculate gain in body weight at respective fortnight and the data obtained was used to compare the treatment performance. The fortnightly body weight gains in goats for different groups are presented in Table 4.8 and depicted graphically in Fig. 4.5.

Table 4.8 Average fortnightly body weight gain in experimental groups (kg)

Fortnights	Groups			
	T_0	T_1	T_2	T_3
1st Fortnight	$0.82^{Aa}\pm0.03$	$0.89^{Aa}\pm0.10$	$0.91^{ABa}\pm0.03$	$1.02^{Bab}\pm0.05$
2nd Fortnight	$0.96^{Aab}\pm0.03$	$0.99^{Aab}\pm0.11$	$1.00^{Aab}\pm0.04$	$1.22^{Bbc}\pm0.23$
3rd Fortnight	$1.08^{ABb}\pm0.02$	$1.00^{Aab}\pm0.09$	$1.11^{Babc}\pm0.03$	$1.21^{Cbc}\pm0.10$
4th Fortnight	$1.08^{Ab}\pm0.03$	$1.10^{ABbc}\pm0.10$	$1.11^{Bbc}\pm0.03$	$1.18^{Bbc}\pm0.15$
5th Fortnight	$1.20^{Abc}\pm0.04$	$1.21^{Ac}\pm0.08$	$1.13^{Abc}\pm0.05$	$1.37^{Bc}\pm0.14$
6th Fortnight	$1.19^{Bbc}\pm0.07$	$1.05^{Abc}\pm0.09$	$1.16^{ABbc}\pm0.03$	$1.11^{ABabc}\pm0.13$
7th Fortnight	$1.26^{ABc}\pm0.04$	$1.15^{Ac}\pm0.09$	$1.21^{ABc}\pm0.03$	$1.26^{Bc}\pm0.09$
8th Fortnight	$1.03^{Ab}\pm0.04$	$1.15^{BCc}\pm0.10$	$1.07^{ABbc}\pm0.02$	$1.18^{Cbc}\pm0.16$
Pooled Mean	$1.08^{A}\pm0.02$	$1.07^{A}\pm0.03$	$1.09^{A}\pm0.02$	$1.20^{B}\pm0.05$

^{a,b,c} –Means bearing different superscript in a column differ significantly ($p<0.01$), CD -0.15
^{A,B,C} - Means bearing different superscript in a row differ significantly ($p<0.01$), CD- 0.10
NS– Non significant

The fortnightly body weight gains were not consistent and revealed no specific trend. The fluctuations in the body weight gain could be due to adverse seasonal changes and marginal variations in the age of experimental goats. The pooled mean for fortnightly body weight gain (kg) differed significantly ($p<0.05$) and was higher in T_3 (1.20±0.05) group as compared to other treatment groups, whereas, it was found to be comparable in T_2 (1.09±0.02), T_0 (1.08±0.02) and T_1 (1.07±0.03) groups. The significant differences for gain in body weight existed at all fortnightly interval and in general T_3 treatment group exhibited consistently higher gain in body weight in comparison to T_0, T_1 and T_2 groups. Except at 8th

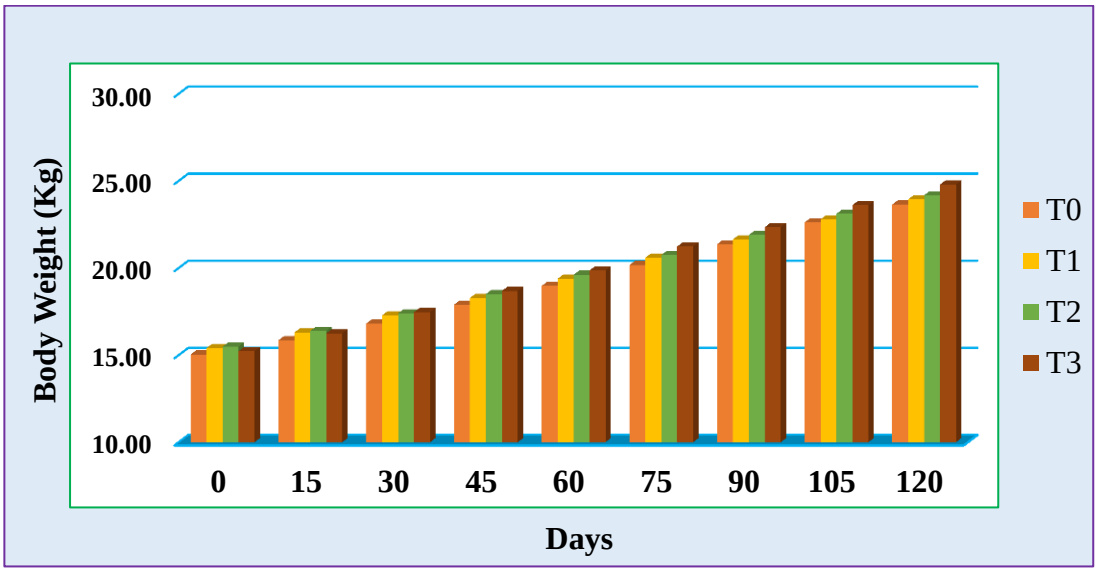


Figure 4.4 Average fortnightly body weight in experimental goats (kg)

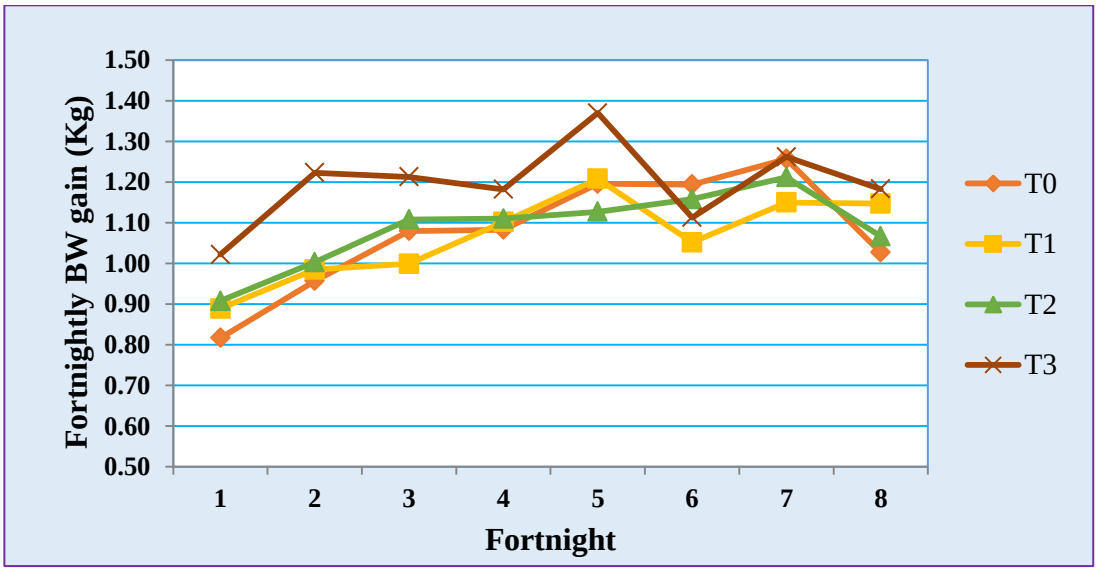


Figure 4.5 Average fortnightly body weight gain in experimental groups (kg)

fortnight, the body weight gains in T_1 and T_2 groups at fortnightly interval were found to be comparable with control (T_0) group. Thus, T_3 dietary group with cotton stalk treated with liquid enzyme and water @ 500 ml/kg stalk for 6 h revealed better in body weight gain than other groups. The pre-treatment of shredded cotton stalk with enzyme solution at the rate of 500 ml/kg and incubation for 6 h may have created favorable rumen fermentation, thereby utilization of nutrients and culminated into positive body weight gain in goats from T_3 group.

The present findings are parallel with Ingle (2021) who reported that the average body weight gain was increased in gram straw based pelleted complete feed supplemented with fiber degrading enzymes and yeast than that of control which indicated better effect of pelleting on body weight gain and fortification with enzymes and yeast further elevated the body weight gain. He opined that supplementation of enzymes and yeast may have created favorable rumen fermentation, thereby utilization of nutrients and culminated into positive growth in goats. Further, Thorat (2021) reported similar fortnightly body weight gains on complete replacement of gram straw with cotton stalk. However, the body weight gains were not consistent throughout the experimental period, rather it was not linear and no periodic relationship could be established in body weight gains when cotton stalk based pelleted complete feed supplemented with NSP enzymes and yeast in growing goat.

4.4.4 Average daily gain in body weight

The average daily gain in body weight was calculated at respective fortnight and was used for comparing the different treatment groups. The average daily gains (ADG) in goats for different groups are presented in Table 4.9 and depicted graphically in Fig 4.6.

Table 4.9 Average daily gain of goats in experimental goats (g)

Fortnights	Groups			
	T_0	T_1	T_2	T_3
1st Fortnight	$54.44^{Aa}\pm2.17$	$59.28^{Aa}\pm6.44$	$60.56^{Aa}\pm2.04$	$68.11^{Ba}\pm3.43$
2nd Fortnight	$63.78^{Aab}\pm1.79$	$65.67^{Aab}\pm7.09$	$66.89^{Aab}\pm2.85$	$81.56^{Bbc}\pm15.02$
3rd Fortnight	$72.00^{Abc}\pm1.47$	$66.61^{Aabc}\pm6.21$	$73.89^{ABbc}\pm2.07$	$80.89^{Bb}\pm6.83$
4th Fortnight	$72.22^{Abc}\pm2.27$	$73.44^{Abcd}\pm6.51$	$74.00^{Abc}\pm2.28$	$78.78^{Ab}\pm9.89$
5th Fortnight	$79.72^{Acd}\pm2.89$	$80.56^{Ad}\pm5.67$	$75.11^{Abc}\pm3.15$	$91.33^{Bc}\pm9.27$
6th Fortnight	$79.61^{Bcd}\pm4.49$	$70.11^{Abcd}\pm6.01$	$77.22^{ABc}\pm2.21$	$74.22^{ABab}\pm8.61$
7th Fortnight	$83.78^{Bd}\pm2.49$	$76.67^{Acd}\pm6.01$	$80.78^{ABc}\pm1.81$	$84.11^{Bbc}\pm5.74$
8th Fortnight	$68.44^{Ab}\pm2.36$	$76.44^{BCcd}\pm6.56$	$71.11^{ABb}\pm1.53$	$78.89^{Cb}\pm10.56$
Pooled Mean	$71.75^{A}\pm1.57$	$71.10^{A}\pm2.27$	$72.44^{A}\pm1.14$	$79.74^{B}\pm3.16$

$^{a,b,c.d.}$ –Means bearing different superscript in a column differ significantly (P<0.01), CD -10.22
^{A,B,C} - Means bearing different superscript in a row differ significantly (P<0.01), CD- 7.23
NS– Non significant

The ADG differed statistically between groups at every fortnight except during 4th fortnight. The variations in ADG were observed at different fortnight in almost all treatment groups but definite trend of increase or decrease could not be established. Apparently, it appears from the observations that the ADG was found to be increased till 5th fortnight and then gradual decline was noticed in ADG. The pooled mean for average daily gain was found to be significantly ($p<0.01$) higher in T_3 (79.74±3.16 g/day) group than T_0, T_1 and T_2 group. However, the pooled mean for ADG was statistically comparable amongst T_0, T_1 and T_2 groups with ADG of 71.75±1.57, 71.10±2.27 and 72.44±1.44 g, respectively. Thus, from the findings in present study, it was evident that pretreatment of cotton stalk with fibrolytic enzymes at higher level of dose had comparatively better ADG. This could be attributed to improve breakdown of ligno-cellulosic bond, releasing more soluble fiber fraction for digestion and improvement in nutrient availability for growth.

The ADG reported in the study was consistent with Salem *et al.* (2011) who observed that ADG differed significantly in enzymes treated than untreated complete feed in goat because of an increase in the available nutrients to the

animals for deposition and growth. Ingle (2021) reported that the average daily gain was increased in gram straw based pelleted complete feed supplemented with fiber degrading enzymes and yeast in goats.

The results are contrary to Torres *et al.* (2013) who concluded that ADG was lesser by using different dose of enzymes (5 and 7.5 ml/kg DM of TMR) than control (TMR) and Kewan *et al.* (2019) who reported decrease ADG in Moringa tree stalk treated fungi (139g/day) and yeast (146 g/day) complete feed as compared to control (173 g/day) in ADG.

Shembekar (2019) reported ADG of 105.74 g/day in 50% ozonated cotton stalk based pelleted complete feed, which was higher than the present findings. Mousa *et al.* (2022)[b] evaluated that average weight gain (g) were increased ($p \leq 0.05$) in G3 (TMR containing 60 % concentrate mixture + 20% EC + 20% WS supplemented with 1kg/ton diet of Calfo-Care) lambs compared with G1(TMR containing 60 % concentrate mixture + 20% EC + 20% WS) lambs while, G2 (TMR containing 60 % concentrate mixture + 20% EC + 20% WS supplemented with 0.5 kg/ton diet of Calfo-Care) recorded intermediate values due to combination of both fibrolytic enzymes and probiotics to improve the growth performance and health of fattening lambs.

4.4.5 Average daily dry matter intake (DMI) at fortnightly interval in experimental goats

The daily dry matter intake (DMI) in goats for different groups was calculated at fortnightly interval and the data obtained are presented in Table 4.10 and depicted graphically in Fig 4.7.

Table 4.10 Average daily dry matter intake at fortnightly interval in experimental goats (g)

Fortnights	Groups			
	T_0	T_1	T_2	T_3
1st Fortnight	535.35^{a}±73.96	508.29^{a}±56.71	533.76^{a}±55.54	510.96^{a}±76.12
2nd Fortnight	587.48^{ab}±72.42	570.97^{ab}±58.44	587.19^{ab}±53.84	560.26^{ab}±77.64
3rd Fortnight	628.95^{abc}±69.27	606.66^{abc}±55.94	637.85^{ab}±52.21	614.96^{abc}±78.24
4th Fortnight	678.38^{bcd}±68.43	650.04^{bcd}±52.74	660.24^{bcd}±50.50	651.90^{bcd}±76.22
5th Fortnight	708.57^{cd}±64.74	694.03^{cd}±55.88	688.79^{bcd}±49.24	700.48^{cde}±74.39
6th Fortnight	724.30^{cd}±62.70	714.58^{cd}±57.24	715.14^{cd}±46.73	752.39^{de}±76.61
7th Fortnight	755.71^{d}±62.46	741.04^{d}±58.50	748.45^{d}±54.14	792.02^{e}±80.25
8th Fortnight	763.34^{d}±60.38	768.50^{d}±60.42	804.09^{d}±60.91	835.98^{e}±81.66
Pooled Mean	672.76±24.54	656.77±22.26	671.94±20.98	677.37±29.70

$^{a,b,c.d.}$ *–Means bearing different superscript in a column differ significantly (p<0.01), CD =106.11*

The observations of average dry matter intake recorded at fortnightly interval did not reveal any significant difference in average DMI between the treatment groups at respective fortnight. However, DMI varied significantly ($p<0.01$) in treatment groups at different fortnights. The average data for dry matter intake (DMI) were 672.76±24.54, 656.77±22.26, 671.94±20.98 and 677.37±29.70 g/day in T_0, T_1, T_2 and T_3 groups, respectively. For each group, the daily DMI was found to be increased with increase in age which could be related to increased body weights at every fortnight with concomitant increase in dry matter requirement for satisfying additional nutrient requirement of the body. The pooled mean of dry matter intake though was comparable in all experimental groups, numerically higher (677.37±29.70 g/day) was noticed in T_3 group as compared to other groups. As such, the palatability and acceptability of cotton stalk was good in pelleted complete feed of goats and did not reveal any adverse effect of enzyme treatment of cotton stalk on the voluntary dry matter intake.

The present findings of DMI (g/d) were similar with Salem *et al.* (2011) and Torres *et al*. (2013) when fed with complete feed with and without supplementation of enzymes (cellulase, xylanase, alpha amylase and protease) and enzymatic extract of *Cellulomonas flavigena* with @ 0,5 and 7.5 ml/kg DM of TMR in goat and lambs, respectively. Kholif *et al.* (2017)[b] stated that DMI

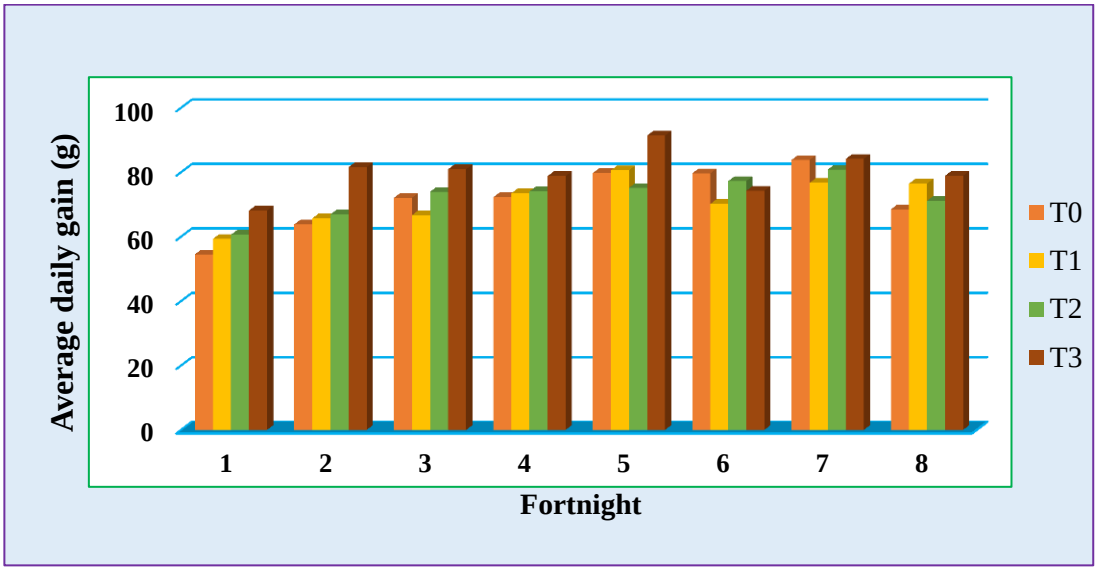


Figure 4.6 Average daily gain of goats in experimental goats (g)

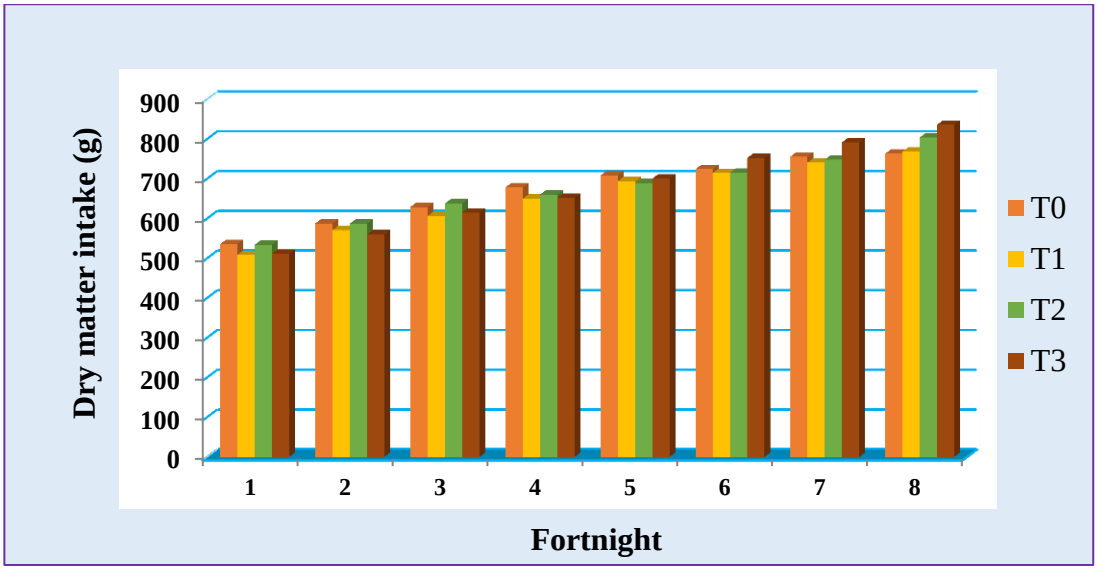


Figure 4.7 Average daily dry matter intake at fortnightly interval in experimental goats (g)

(kg/head/day) was comparable between treatment supplemented with fibrolytic enzymes (2 and 4gm/head/day) than control (alpha alpha hay and concentrate feed mixture). Mahrous *et al.* (2019)[a] suggested that DMI (g/d) was similar in treatment (CFM + olive tree by product treated with biological EM1 and CFM + olive tree by product treated with molasses + urea + minerals + vitamins) and control (CFM + olive tree by product group in sheep.

The present findings are contrary to Burghate (2021) who revealed that the dry matter intake was comparatively lesser when gram straw was replaced with ozone treated cotton stalk either at 75% or 100% level in pelleted complete feed further, Thorat (2021) also reported higher DMI when gram straw was replaced with cotton stalk supplemented NSP enzymes and yeast in growing goats

4.4.6 Average percent daily dry matter intake (% BW) of experimental goats

The daily dry matter intake in relation to body weight was calculated and expressed as percent body weight. The calculated percent daily dry matter intake (% BW) is presented in below in Table 4.11 and depicted graphically in Fig 4.8.

Table 4.11 Average daily dry matter intake (% body weight) in experimental goats

Fortnights	Groups			
	T_0	T_1	T_2	T_3
1st Fortnight	$4.68^{ab}\pm0.12$	$4.41^{a}\pm0.18$	$4.53^{a}\pm0.09$	$4.44^{a}\pm0.08$
2nd Fortnight	$4.87^{bcd}\pm0.10$	$4.68^{bc}\pm0.20$	$4.72^{ab}\pm0.07$	$4.55^{ab}\pm0.11$
3rd Fortnight	$4.91^{cd}\pm0.07$	$4.71^{bc}\pm0.20$	$4.84^{b}\pm0.07$	$4.67^{ab}\pm0.10$
4th Fortnight	$5.01^{d}\pm0.06$	$4.79^{bc}\pm0.26$	$4.72^{ab}\pm0.08$	$4.68^{abc}\pm0.12$
5th Fortnight	$4.93^{cd}\pm0.06$	$4.80^{c}\pm0.23$	$4.66^{ab}\pm0.08$	$4.72^{bc}\pm0.14$
6th Fortnight	$4.77^{abcd}\pm0.08$	$4.70^{bc}\pm0.21$	$4.59^{a}\pm0.08$	$4.82^{c}\pm0.17$
7th Fortnight	$4.70^{abc}\pm0.08$	$4.62^{bc}\pm0.19$	$4.54^{a}\pm0.12$	$4.78^{c}\pm0.15$
8th Fortnight	$4.54^{a}\pm0.09$	$4.55^{ab}\pm0.16$	$4.65^{ab}\pm0.14$	$4.81^{c}\pm0.16$
Pooled Mean	4.80±0.03	4.66±0.07	4.66±0.03	4.68±0.05

$^{a,b,c.d.}$ –Means bearing different superscript in a column differ significantly ($p<0.01$), CD– 0.24

The treatment means for daily matter intake as percent of body weight did not differ significantly and so were comparable at all the fortnights. The pooled mean of DMI percent body weight for T_0, T_1, T_2 and T_3 groups were arrived to be 4.80±0.03, 4.66±0.07, 4.66±0.03 and 4.68±0.05, respectively and were comparable in all treatment groups. The fortnightly DMI percent BW showed significant ($p<0.01$) differences from 1st to 8th fortnight in all treatment groups. The DMI on percent body weight basis was optimum and as per the physiological range of dry matter requirement of goats, however goats consumed more dry matter when offered cotton stalk based pelleted complete feed given pretreatment with fibrolytic enzymes. It was thus revealed that experimental goats adjusted their dry matter intake according to body weight and DMI percent body weight was optimum.

The present findings corroborate with Thorat (2021) who stated 4.18 kg DMI percent BW in cotton stalk based pelleted complete feed with supplementation of enzymes and yeast in growing goats. Mousa *et al.* (2022)[b] evaluated that DMI percent body weight were comparable between G3 (TMR containing 60 % concentrate mixture + 20% EC + 20% WS supplemented with 1 kg/ton diet of Calfo-Care), G1 (TMR containing 60 % concentrate mixture + 20% EC + 20% WS) and G2 (TMR containing 60 % concentrate mixture + 20% EC + 20% WS supplemented with 0.5 kg/ton diet of Calfo-Care) of fattening lambs.

The present findings are contrary to Ingle (2021) who stated lower DMI (% BW) as 3.96 kg in enzymes and yeast supplemented gram straw based pelleted complete feed. Burghate (2021) reported comparatively lesser DMI percent body weight than present study when gram straw was replaced with ozone treated cotton stalk with 75% or 100% level in pelleted complete feed supplemented with NSP enzymes and yeast.

4.4.7 Average fortnightly FCR

The feed conversion ratio (FCR) is always considered to be a real index of efficiency of feed utilization for the body purposes. The FCR in relation to growth

was calculated in present study and the findings are presented in Table 4.12 and depicted graphically in Fig 4.9.

Table 4.12 Average FCR in experimental goats

Fortnights	Groups			
	T_0	T_1	T_2	T_3
1st Fortnight	$9.05^{Ca}\pm0.94$	$8.00^{Ba}\pm0.18$	$8.15^{Ba}\pm0.63$	$7.07^{Aa}\pm0.85$
2nd Fortnight	$8.49^{Ba}\pm0.83$	$8.14^{Ba}\pm0.22$	$8.17^{Ba}\pm0.61$	$6.52^{Aa}\pm1.02$
3rd Fortnight	$8.08^{Ba}\pm0.73$	$8.51^{Bab}\pm0.22$	$8.04^{Ba}\pm0.54$	$7.20^{Aa}\pm0.60$
4th Fortnight	$8.71^{Ba}\pm0.67$	$8.29^{ABa}\pm0.22$	$8.35^{Ba}\pm0.62$	$7.53^{Aa}\pm0.69$
5th Fortnight	$8.24^{Ba}\pm0.49$	$8.03^{Ba}\pm0.24$	$8.65^{Ba}\pm0.77$	$7.18^{Aa}\pm0.74$
6th Fortnight	$8.45^{Aa}\pm0.39$	$9.55^{Bb}\pm0.29$	$8.63^{Aa}\pm0.45$	$8.91^{ABbc}\pm0.60$
7th Fortnight	$8.37^{Aa}\pm0.46$	$9.05^{Aab}\pm0.37$	$8.64^{Aa}\pm0.58$	$8.80^{Abc}\pm0.96$
8th Fortnight	$10.37^{Bb}\pm0.56$	$9.45^{Ab}\pm0.46$	$10.53^{Bb}\pm0.68$	$9.42^{Ac}\pm0.97$
Pooled Mean	$8.72^{B}\pm0.24$	$8.63^{B}\pm0.13$	$8.64^{B}\pm0.23$	$7.83^{A}\pm0.30$

^{A,B,C}–Means bearing different superscript in a row differ significantly, CD group– 0.786 ($p<0.01$)
^{a,b} –Means bearing different superscript in a column differ significantly, CD periods – 1.11 ($p<0.01$)

The feed conversion ratio in almost all treatment groups gradually declined with increasing age exhibiting higher dry matter intake per unit gain in body weight. The FCR was not even consistent during the succeeding fortnights; however, it apparently revealed that FCR was quite better in earlier 1st to 5th fortnight than latter fortnights of experiments. During the entire period of study, T_3 group showed superior FCR as compared to other treatment groups, though it was comparable with other groups at 5, 6, 7 and 8th fortnight. The pooled FCR varied significantly between the groups and it was significantly ($p<0.05$) superior in T_3 (7.83 ± 0.30) whereas, it was comparable for T_0 (8.72 ± 0.24), T_1 (8.63 ± 0.13) and T_2 (8.64 ± 0.23) groups, indicating improved feed efficiency in T_3 group receiving pelleted feed with inclusion of cotton stalk treated with E_{500} treatment with incubation for 6 hours. The findings revealed that pretreated cotton stalk showed better feed conversion ratio than control group, indicated better utilization of enzyme treated cotton stalk in pelleted complete feed and better nutrient availability especially fiber fractions due to pre-treatment with fibrolytic enzymes.

The results corroborates with Mahrous (2005) who reported that groups fed ration containing treated wheat straw (*Trichoderma viride* + *T. reesei*) or cotton stalk treated *T. viride* or *Cheatomium cellublticum* or treated with fungi revealed better ($p<0.05$) in FCR as DM. Salem *et al* (2011) evaluated that feed efficiency was better in enzymes addition group (cellulase, xylanase, amylase and protease) compared to control because of cellulase feeding on feed intake and digestibility. of *Cellulomonas flavigens* on productive performance. Mahrous *et al.* (2019)[a] concluded that feed conversion efficiency was best in R_2 (CFM + olive tree by products with biological treatment) followed by R_3 (CFM+ olive tree by product treated with 91% molasses, 2.5% urea and 6.5% mixing minerals and vitamins) as compared to control (CFM+ olive tree by products). The improvement of FCR may be due to improvement in both nutrient digestibilities and nutritive value of feed. Jadhav (2019) and Shembekar (2019) also reported similar FCR when gram straw was replaced with cotton stalk at 25 percent level without and with ozone treatment, respectively than present findings.

The present findings are contrary to Torres *et al.* (2013) who showed less effect on feed conversion efficiency at different doses (0, 5 and 7.5 ml/kg DM of TMR) enzymatic extract of *Cellulomonas flavigena*.

4.4.8 Digestibility of nutrient (%)

The digestibility trial was conducted during last five days of the experiment and digestibility coefficients of various nutrients were determined. The data obtained on different nutrients are presented in Table 4.13 and depicted graphically in Fig 4.10

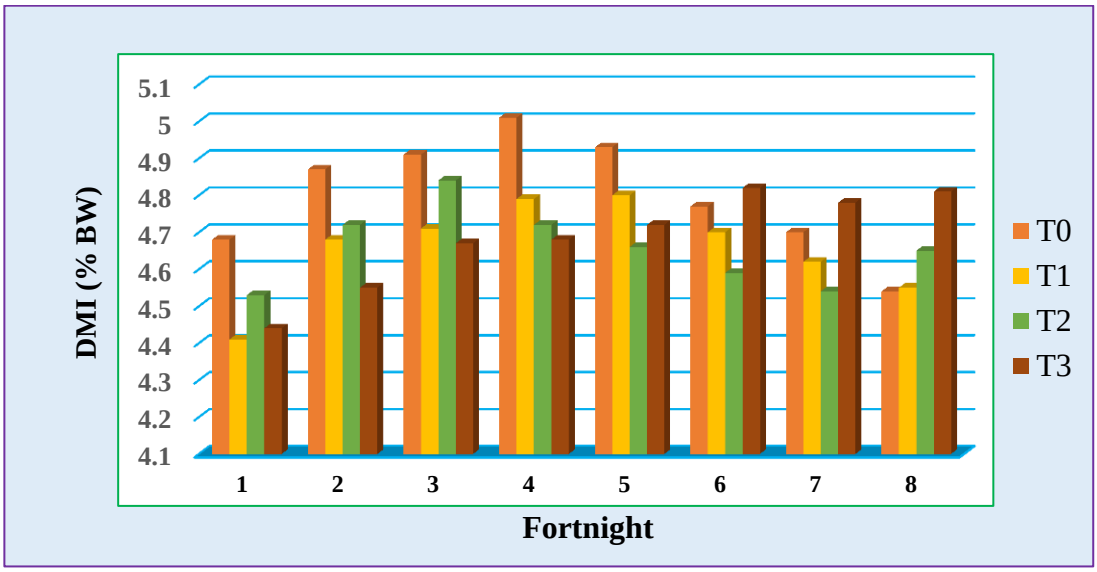


Figure 4.8 Average daily dry matter intake (% body weight) in experimental goats

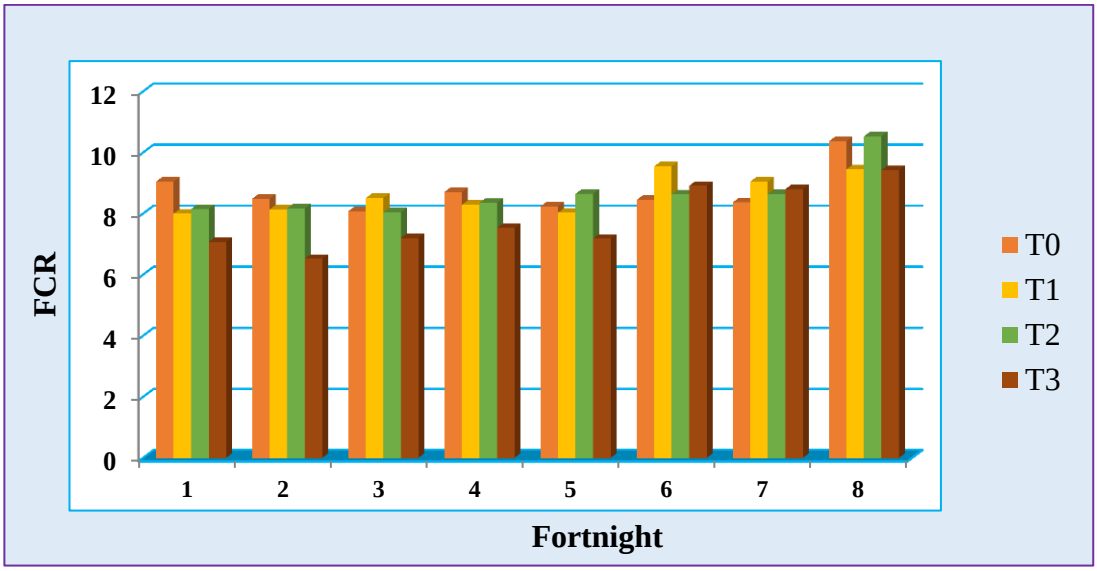


Figure 4.9 Average FCR in experimental goats

Table 4.13 Nutrient digestibility (%) in experimental goats

Cellulose	78.23±2.95	85.14±4.42	85.86±2.42	84.33±2.23	NS

NS- Non-significant,
*^{A,B,C}–Means bearing different superscript in a row differ significantly *($p<0.05$)*

The dry matter digestibility in T_0 (control), T_1, T_2 and T_3 group was found to be 54.29±2.58, 53.67±1.25, 59.48±2.39 and 55.31±2.89 percent, respectively. The DM digestibility though was higher in T_2 and T_3 group over the control and T_1 group, the differences amongst the groups however were found to be non-significant. The organic matter digestibility in T_0 (control), T_1, T_2 and T_3 group was found to be 54.51±2.53,54.11±1.29, 59.19±2.43 and 56.47±2.71 percent, respectively and the differences amongst the group were non-significant. The results thus revealed better DM and OM digestibility in T_2 group as compared to other groups. However crude protein digestibility differed significantly ($p<0.05$) and it was highest in T_2 (75.37±1.98) followed by T_3 (74.69±0.82), T_0 (control) 70.61±1.64 and lowest in T_1 (70.24±0.79). The EE and NFE digestibility did not vary significantly. The crude fiber digestibility differed significantly ($p<0.05$) and was highest in T_3 group (47.36±3.29) followed by T_2 group (43.53±4.53) and lowest but comparable values between in T_0 (33.97±3.13) and T_1 (35.73±3.59) groups.

The NDF and ADF digestibility was observed to be significantly ($p<0.05$) higher in T_2 group (56.54± 1.93) followed by T_3 (54.68± 2.41), T_1 (50.82±1.91) and lower in T_0 group (48.28±0.87) and T_2 (50.82±3.21) followed by T_3 (48.59±3.99), respectively. Thus significantly better digestibility was evident for NDF and ADF fractions due to pre-treatment with fibrolytic enzymes as compared to the control. The cellulose and hemicellulose digestibilities however could not reveal statistical differences.

The increased fiber digestibility noticed in present study can be related to pretreatment of cotton stalk used as sole roughage in the pelleted complete feed with fibrolytic enzyme solution. The improvement in most nutrient digestibility parameters in this study could be explained on the basis that dietary supplementation with cellulase and xylanase may have enhanced the ruminal

microbial activity and communities, thus increasing the gut health and ecology through rumen maturity by favoring microbial establishment, increasing the fiber digestion of feedstuff, reducing the fluid viscosity and ruminal ammonia, and improving the concentration of volatile fatty acids in the rumen (Jiang *et al.,* 2020).

The improvements in digestibilities are associated with increasing digestion in fibrous materials in addition to the increased bacterial digestion of cell wall contents. Improved performance might be due to increase in digestibility which yields more energy and/or nutrient availability to rumen microbes because of enzyme treatment. Fibrolytic enzymes contain a wide variety of polysaccharidase enzymes that solubilize fiber and provide some essential nutrients or growth factors to rumen microorganisms. Due to this there was a marked effect on increasing the total microbial population in the rumen. Pretreatment with fibrolytic enzymes, such as cellulase and xylanase, to feeds can reduce the feed viscosity, which increases the absorption of nutrients, and liberates nutrients either through the hydrolysis of non-biodegradable fibers (Azzaz *et al.,* 2021; Mousa *et al.,* 2022[a]).

The observation of increased CP digestibility particularly in T_2 and T_3 group can be illustrated on the basis that lignocellulosic enzymes like cellulases and xylanases have hydrolytic effect. They are often utilized to extract or enhance the protein content of biomass. These enzymes are used for the pre-treatment purpose in protein recovery process. The probable mechanism of action behind increased protein digestibility witnessed in ruminants is the breakdown of complex fibrous network and the release of protein from the interlinked matrix of cellulose, hemicellulose and lignin. (Zulkarnain *et al.,* 2016). Further, the enzymatically pre-digested animal feed may have helped in promoting probiotic strains inside the ruminant gut creating a favorable environment for improved protein digestibility (Saini *et al.,* 2022).

As like in present study, the dry matter and crude protein soluble fractions of the higher fiber diet were found to be increased due to exogenous enzymes addition by Alvarez *et al. (*2009). The findings of (Jiang *et al.,* 2020) are parallel

to present study with regard to fiber digestibility. Even, (Arriola *et al.,* 2017) made similar observations and noticed positive effects of fibrolytic enzymes (xylanases and cellulases) in ruminant diets on total-tract digestibility of DM and NDF in dairy cows.

The research findings in present study are also in tune with Salem *et al.* (2011) and Ingle (2021) who reported increased CP and NDF digestibility by addition of enzymes in goats.

The findings in present study can also be correlated to Beauchemin *et al.* (2003) who mentioned that the main effect of EFE is increased enzymatic activity inside the rumen, which increased digestibility of the total diet fed and further suggested that, the increase in digestibility are not limited to the dietary component to which the enzymes are used, which suggest why fibrolytic enzymes can be effective when supplemented to the concentrate portion of the diet. Increment hydrolytic capacity of the rumen can also lead to an increase in digestibility of the non-fiber carbohydrate fraction, in addition to increasing digestibility of the fiber components of a diet; they suggest that fibrolytic enzymes can be effective in high-concentrate diets.

The digestibility results suggest that addition of exogenous enzymes help to remove phenolic barrier that limit the microbial digestion of the cell wall and perhaps improve the colonization of food particles by ruminal bacteria. It has been suggested that greater benefits would be obtained using combination of fibrolytic enzymes (Eun and Beauchemin, 2007). The variation of responses of fibrolytic enzymes treatment / supplementation could be attributed to the retention time of different types of fiber in the rumen, exposure time of fiber to the fibrolytic enzymes process, rate of particle size reduction, particle density and rate of digestion.

4.8.9 Rumen liquor pH of goats in different groups

The average rumen liquor pH values are presented in Table 4.14 and depicted graphically in Fig 4.11.

Table 4.14 Rumen liquor pH of goats in different groups

Periods	Groups			
	T_0	T_1	T_2	T_3
0^{th} day	$6.40^{a}\pm0.03$	$6.41^{a}\pm0.04$	$6.40^{a}\pm0.06$	$6.43^{a}\pm0.06$
30^{th} day	$6.60^{b}\pm0.07$	$6.60^{b}\pm0.05$	$6.65^{b}\pm0.07$	$6.61^{b}\pm0.07$
60^{th} day	$6.68^{Ab}\pm0.04$	$6.75^{ABc}\pm0.05$	$6.78^{Bc}\pm0.04$	$6.81^{Bc}\pm0.04$
90^{th} day	$6.85^{ABc}\pm0.04$	$6.81^{Acd}\pm0.03$	$6.86^{ABc}\pm0.02$	$6.93^{Bc}\pm0.05$
120^{th} day	$6.86^{c}\pm0.06$	$6.86^{d}\pm0.05$	$6.86^{c}\pm0.04$	$6.91^{c}\pm0.04$
Pooled Mean	6.68±0.04	6.69±0.03	6.71±0.03	6.74±0.04

$^{a,b,c.}$ –Means bearing different superscript in a column differ significantly (p<0.01), CD -0.10
^{A,B,C} - Means bearing different superscript in a row differ significantly (p<0.01), CD- 0.09
NS– Non significant

The average rumen liquor pH values for different groups were 6.68±0.04, 6.69±0.03, 6.71±0.03 and 6.74+0.04, respectively. The rumen liquor pH was comparable for all groups on 0^{th} day, however it was increased at successive month in each group and tends to be constant statistically on 90^{th} day onwards. The rumen liquor pH values were quite less at the start of experiment, whereas it has reached to the normal after feeding of experimental diet to the goats and has created positive ruminal environment for optimum fermentation. The pH values of

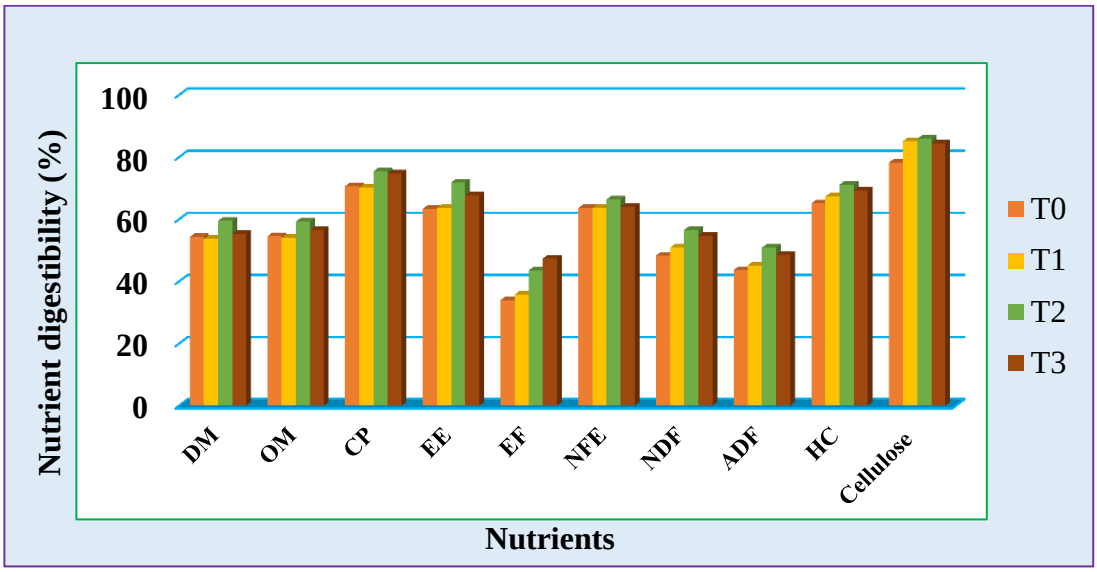


Figure 4.10 Nutrient digestibility (%) in experimental goats

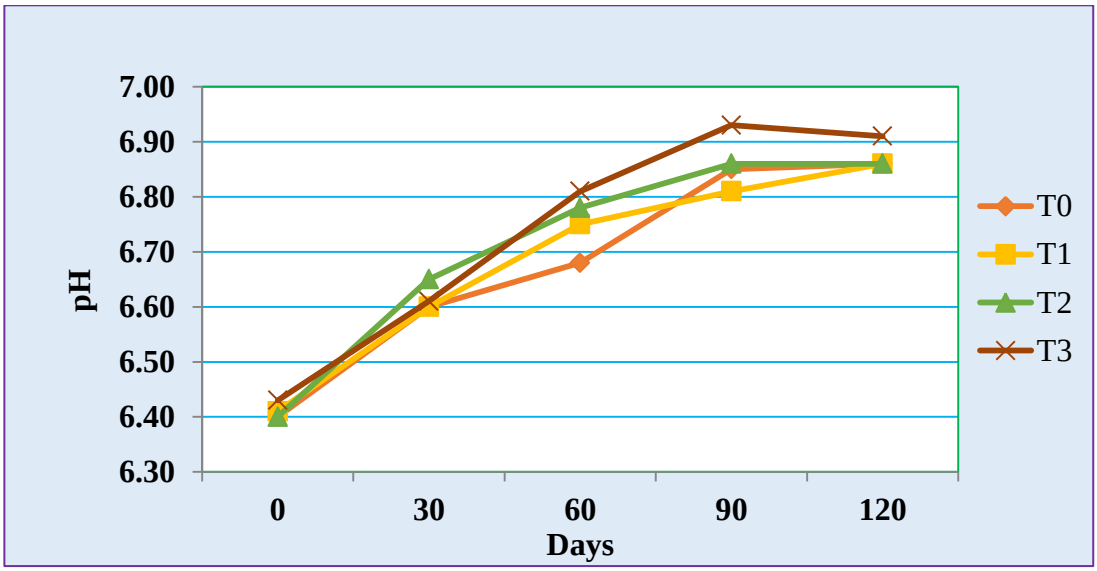


Figure 4.11 Rumen liquor pH of goats in different groups

strained rumen liquor observed in the experiment at monthly interval in all dietary groups were within the physiological range (Chakrabarti, 2006). Rumen pH is an important indicator that reflects rumen function and stability of the intra ruminal milieu and present findings revealed that, the ruminal pH was not influenced due to the treatment of cotton stalk as roughage source in pelleted complete feed of growing goats and also due to enzyme supplementation.

Similar observations were recorded by Ganai *et al*. (2011) who evaluated that the effect of fibrolytic enzymes supplemented to bajra straw based complete feed and did not find altered rumen pH (6.84 in control and 6.80 in treatment). Similar findings were also observed by Kewan *et al*. (2019) with the postulations that *Trichoderma reesei* fungus and *Saccharomyces cerevisiae* yeast degrades native cellulose utilizing a set of cellulolytic enzymes dominated by two cellobiohydrolases i.e CBHI and CBHII which causes synergistic action which resulted in unaltered pH. The findings are consistent with Shembekar (2019), Jadhav (2019) and Thorat (2022) using cotton stalk in pelleted complete feed of goats. The results for pH on enzyme supplementation are also supported by Marwan *et al.* (2019) who recorded non-significant difference in pH 6.83 (control) and 6.93 for treated basal ration (consisting of concentrate feed mixture, wheat straw, berseem and zymogen liquid @ 12 ml per 100 kg animal weight per head) and by Almaamory *et al.* (2020) who found non-significant difference in pH in control (6.88) fed the basal diet of concentrate and green alfalfa and basal diet plus 0.5 g of exogenous enzyme labazyme group 6.97 and 6.72 in basal diet having 0.5 g exogenous enzymes Bio SB-Gold group.

On the contrary Torres *et al.* (2013) reported higher pH values on enzymatic extraction from *Cellulomonas flavigena* @ 0, 5.0 and 7.5 ml of extract per kg DM as 7.0, 6.8 and 6.9, respectively, might be attributed to increase in acetate and propionate ratio due to increase in the dose of enzymes than used in the present study which was greater than present value. However, Burghate (2021) recorded less pH (6.48) on ozone treated cotton stalk based pelleted complete feed in goat, which has culminated into less fiber digestibility, as

evident from her study since fiber digestion is optimum when pH is normal (6.6-6.8).

4.8.10 Rumen liquor NH_3-N (mg/100 ml SRL) of goats in different groups

The average rumen liquor NH_3-N values are presented in Table 4.15 and depicted graphically in Fig 4.12.

Table 4.15 Rumen liquor NH_3-N (mg/100 ml SRL) of goats in different groups

Periods	Groups			
	T_0	T_1	T_2	T_3
0th day	25.90^{Bc}±1.06	24.26^{ABc}±0.78	23.33^{Ac}±0.86	23.80^{Ac}±0.80
30th day	20.53^{b}±1.12	20.06^{b}±0.86	20.30^{b}±1.06	19.13^{b}±1.12
60th day	19.36^{ab}±0.84	19.13^{b}±1.47	19.13^{b}±1.38	18.43^{b}±1.16
90th day	19.13^{ab}±0.78	18.90^{b}±1.06	18.66^{b}±1.00	17.73^{b}±1.06
120th day	17.73^{Ba}±1.38	15.86^{ABa}±1.64	15.86^{ABa}±1.68	14.00^{Aa}±1.35
Pooled Mean	20.53^{B}±0.68	19.64^{AB}±0.71	19.46^{AB}±0.68	18.62^{A}±0.74

$^{a,b,c.}$ –Means bearing different superscript in a column differ significantly (p<0.01), CD – 2.13
^{A,B} - Means bearing different superscript in a row differ significantly (p<0.01), CD- 1.90

The rumen liquor NH_3-N varied significantly (p<0.01) between the groups as well as periods. The average NH_3-N values for T_0, T_1, T_2 and T_3 were found to be 20.53±0.68, 19.64±0.71, 19.46±0.68 and 18.62±0.74 mg/100 ml SRL. It is revealed that the rumen liquor NH_3-N production seems to be less after feeding of experimental diet to the goats and favours the positive ruminal environment for optimum fermentation. Inclusion of enzyme treated cotton stalk as roughage source in pelleted complete feed of growing goats culminated into better rumen fermentation. The increment and utilization of rumen ammonia nitrogen concentration by rumen microbes with the fibrolytic enzymes treated pelleted complete feed may be due to higher fermentation rate in fibrolytic enzymes treated pelleted complete feed. It is opined that more feed nitrogen was efficiently utilized by the rumen micro-organisms for the synthesis of microbial protein due to the experimental feed and also state that, the inclusion of enzyme treated cotton stalk in pelleted complete feed of growing goats enhanced the protein degradation

for microbial protein synthesis with less ammonia production by maintaining rumen eco-friendly environment.

The present findings are supported by the observations made by Shembekar (2019), Jadhav (2019), Burghate (2021) and Thorat (2021) on using cotton stalk in pelleted complete feed of goats. Gado *et al.* (2006) also opined that fibrolytic enzymes may improve the stimulation of ruminal microorganism's activity by reducing the NH_3-N concentration in the rumen liquor.

4.8.11 Rumen liquor TVFA (mEq/L SRL) of goats in different groups

The pattern of TVFA values reflect the pattern of fermentation activity in the rumen. The TVFA values obtained in present study are presented in Table 4.16 and depicted graphically in Fig 4.13.

Table 4.16 Rumen liquor TVFA (mEq/L SRL) of goats in different groups

Periods	Groups			
	T_0	T_1	T_2	T_3
0th day	76.00^{Aa}±1.00	82.16^{Ba}±4.49	80.00^{ABa}±1.87	82.33^{Ba}±1.99
30th day	74.00^{Aa}±5.89	81.66^{Ba}±1.87	81.83^{Ba}±7.20	82.83^{Ba}±4.15
60th day	89.50^{Bb}±2.44	85.00^{Aa}±0.36	93.83^{BCb}±3.64	98.66^{Cb}±2.70
90th day	91.00^{Ab}±3.42	91.16^{Ab}±1.92	96.66^{Bb}±1.02	104.83^{Cc}±2.68
120th day	92.00^{Ab}±2.88	92.33^{Ab}±2.56	98.33^{Bb}±0.91	105.66^{Cc}±2.36
Pooled Mean	84.50^{A}±2.05	86.46^{AB}±1.36	90.13^{BC}±2.11	94.86^{C}±2.25

$^{a,b,c.}$ –Means bearing different superscript in a column differ significantly (p<0.01), CD – 5.68
^{A,B,C} - Means bearing different superscript in a row differ significantly (p<0.01), CD- 5.08

The rumen liquor TVFA varied significantly (p<0.01) between the periods and groups. The TVFA values for 0th day were found to be 76.00±1.00, 82.16±4.49, 80.00±1.87 and 82.33±1.99 mEq/L for T_0, T_1, T_2 and T_3 group, respectively. The average TVFA concentration value was significantly (p<0.01) higher for T_3 (94.86±2.25) followed by T_2 (90.13±2.11), T_1 (86.46±1.36) and T_0 (84.50±2.05). The progress of raising ruminal TVFA concentration paralleled with an increase in ruminal pH. The TVFA production tends to be increased at

successive fortnight in the treatment groups as compared to control which has created the favorable ruminal environment.

Mathison and Milligan (1971) indicated that the energetic of rumen microbial production is related to the fermentable materials degraded to TVFA by the rumen microorganisms through digestion. Higher concentration of TVFA in present study supports the theory that the enzyme application in diet resulted in higher ruminal digestion which is confirmed by improvement of DM and NDF degradability (Ganai *et al.,* 2011). The greater amount of TVFA obtained with increase in supplementation of enzyme could have favoured the carbon flow and VFA production and reduce the microbial protein synthesis (Torres *et al.,* 2013).

Similar increasing trend was found by Kholif *et al.* (2017)[b] in treatment group supplemented with fibrolytic enzymes as compared to control group. Marwan *et al.* (2019) explained that increase in TVFA concentration may be due to greater availability of fermentable soluble carbohydrates due to increase in fibrolytic activity in rumen. Similar findings were reported by Shembekar (2019), Burghate (2021) and Thorat (2021) on using cotton stalk supplemented with NSP enzymes and yeast in pelleted complete feed of goats.

The inclusion of treated cotton stalk with liquid fibrolytic degrading enzymes in pelleted complete feed of growing goats may have enhanced the total volatile fatty acids production in the rumen due to reduction in lignin content and corresponding increase in cell contents for fermentation. The observations are supported by Ingle (2021) in growing goats fed with gram straw pelleted complete feed supplemented with NSP enzymes and *Saccharomyces cerevisiae* yeast culture in T_3 group.

4.8.12 Rumen liquor total nitrogen (mg/100 ml SRL) of goats in different groups

The mean rumen liquor total nitrogen values are presented in Table 4.17 and depicted graphically in Fig 4.14.

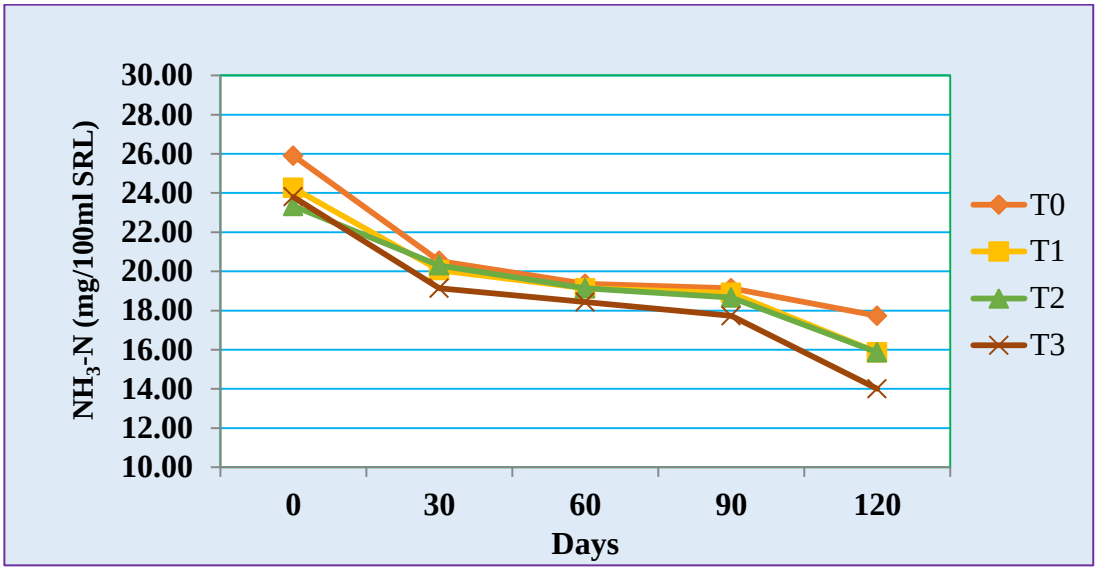


Figure 4.12 Rumen liquor NH3-N (mg/100 ml SRL) of goats in different groups

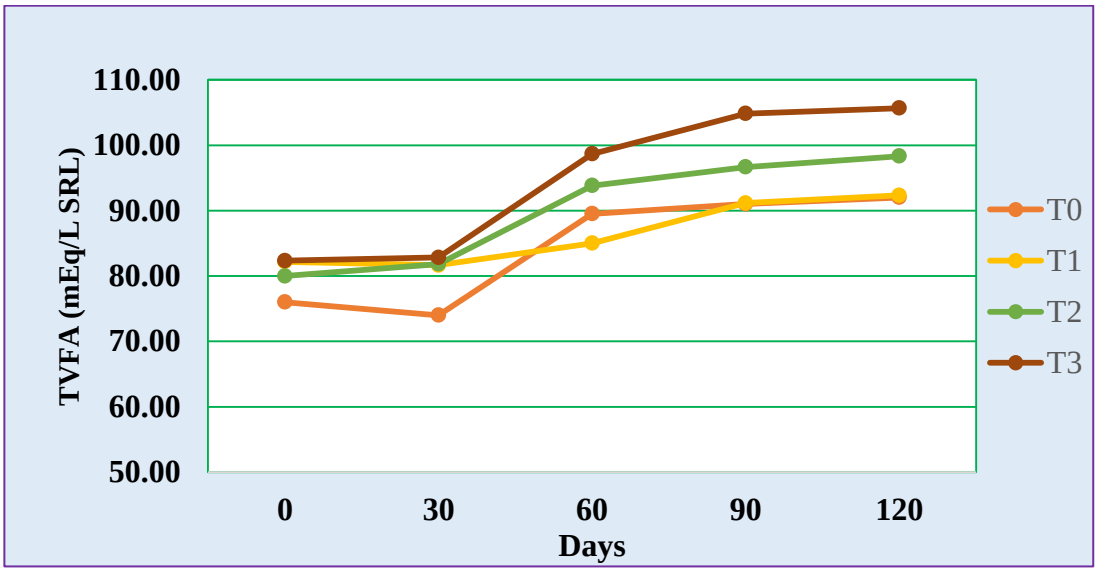


Figure 4.13 Rumen liquor TVFA (mEq/L SRL) of goats in different groups

Table 4.17 Rumen liquor total nitrogen (mg/100 ml SRL) of goats in different groups

Periods	Groups			
	T_0	T_1	T_2	T_3
0^{th} day	93.33±2.95	93.33^{a}±4.66	98.00^{a}±3.61	95.66^{a}±2.33
30^{th} day	100.33±4.30	102.66^{ab}±5.90	102.66^{a}±7.80	107.33^{ab}±4.66
60^{th} day	102.66±10.64	112.00^{bc}±8.08	109.66^{ab}±6.68	112.00^{b}±7.22
90^{th} day	102.66^{A}±12.34	119.00^{Bc}±7.87	116.66^{Bb}±6.92	119.00^{Bb}±5.99
120^{th} day	105.00^{A}±7.87	121.33^{Bc}±2.95	121.33^{Bb}±6.92	137.66^{Cc}±10.48
Pooled Mean	100.80^{A}±3.57	109.66^{AB}±3.22	109.66^{AB}±3.15	114.33^{B}±3.80

$^{a,b,c.}$ –Means bearing different superscript in a column differ significantly (p<0.01), CD – 12.74
^{A,B,C} - Means bearing different superscript in a row differ significantly (p<0.01), CD- 11.39

The rumen liquor total nitrogen production differs significantly (p<0.01) among period and groups. The average total nitrogen values were highest for T_3 (114.33±3.80) and lowest for T_0 (100.80±3.57) group. In present study it is observed that increase in total nitrogen leads to better adaptation of rumen microbes to the pelleted feed in later stages. Increase in total nitrogen may be due to increase in ruminal N fraction and microbial protein by feeding. Decrease in ciliate protozoa can increase microbial nitrogen by up 30% and decrease the methane production up-to 11% because of cellulolytic enzyme action (Kholif and Aziz, 2014 and Newbold *et al.*, 2015).

Shembekar (2019) revealed that, the inclusion of ozone treated cotton stalk in the pelleted complete feed of growing goats could help in the active degradation of protein and hydrolysis of non-protein nitrogenous substances in the feed, keeping healthy rumen environment of goats. The present findings are consistent with Jadhav (2019) and Burghate (2021) who reported similar observations on using cotton stalk in pelleted complete feed of goats and Ingle (2021) reported on using gram straw in pelleted complete feed on growing goats.

4.8.13 Rumen liquor TCA-ppt-N (mg/100 ml SRL) of goats in different groups

The rumen liquor TCA-ppt-N values are presented in Table 4.18 and depicted graphically in Fig 4.15.

Table 4.18 Rumen liquor TCA-ppt-N (mg/100 ml SRL) of goats in different groups

Periods	Groups			
	T_0	T_1	T_2	T_3
0^{th} day	39.66±4.30	39.66±2.33	40.83±5.24	42.00±5.11
30^{th} day	42.00±3.61	42.00±5.11	42.00±0.00	44.33±4.30
60^{th} day	44.33±4.30	42.00±3.61	44.33±6.68	46.66±2.95
90^{th} day	44.33±5.62	46.66±2.95	44.33±7.59	46.66±2.90
120^{th} day	44.33±6.68	49.00±8.66	44.33±4.30	49.00±5.99
Pooled Mean	42.93±2.11	43.86±2.19	43.16±2.27	45.73±2.11

NS - Non significant

The TCA-ppt-N production did not vary significantly between the groups and periodic interval. The average TCA-ppt-N values were found to be 42.93±2.11, 43.86±2.19, 43.16±2.27 and 45.73±2.11 mg/100 ml SRL for T_0, T_1, T_2 and T_3 group. However numerically, higher TCA-ppt-N was observed in T_3 group as compare to T_0 and comparable in T_1 and T_2. This could be due to increased soluble carbohydrate and increase nitrogen intake by growing goats.

Shembekar (2019) revealed numerically more TCA-ppt-N concentrations in goats fed pelleted complete feed containing 25% ozone treated cotton stalk as roughage source (T_1 group) which could be attributed to increased rumen microbial protein synthesis without affecting rumen ecosystem of goats. The present findings are in accordance with Jadhav (2019), Burghate (2021) and Thorat (2021) on using cotton stalk in pelleted complete feed of goats.

4.8.14 Rumen liquor NPN (mg/100 ml SRL) of goats in different groups

The rumen liquor NPN values are presented in Table 4.19 and depicted graphically in Fig 4.16.

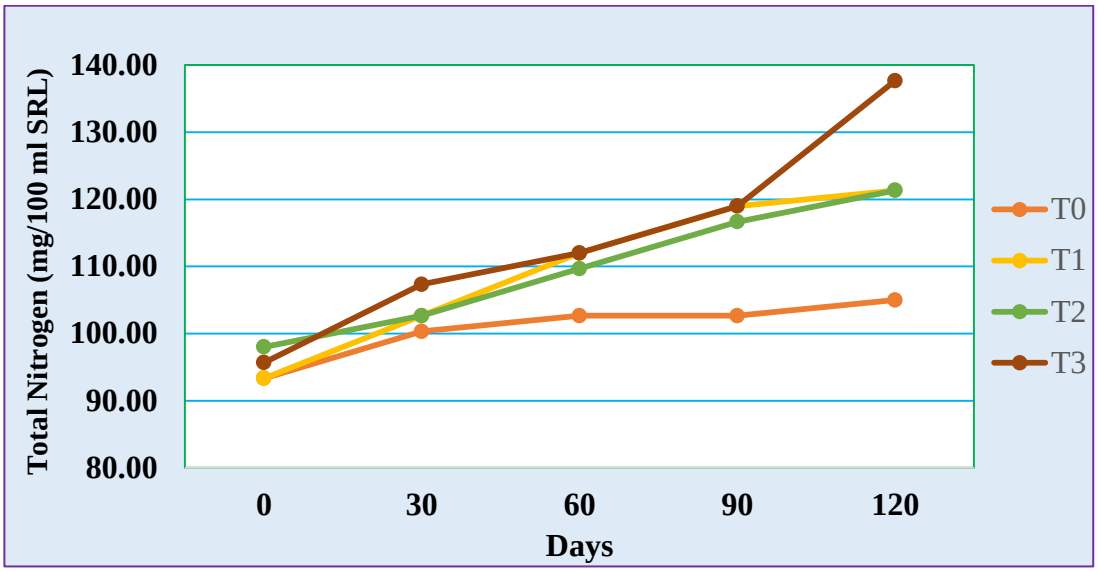


Figure 4.14 Rumen liquor total nitrogen (mg/100 ml SRL) of goats in different groups

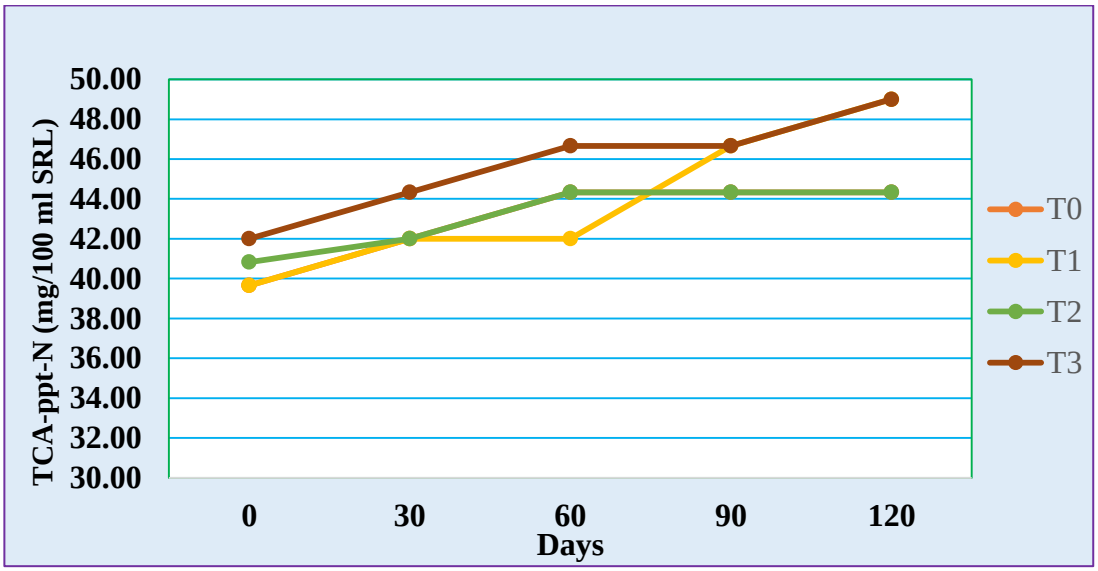


Figure 4.15 Rumen liquor TCA-ppt-N (mg/100 ml SRL) of goats in different groups

Table 4.19 Rumen liquor NPN (mg/100 ml SRL) of goats in different groups

Periods	Groups			
	T_0	T_1	T_2	T_3
0^{th} day	53.66±5.61	53.66^{a}±5.61	57.16^{a}±5.57	53.66^{a}±6.68
30^{th} day	58.33±5.61	60.66^{ab}±8.60	60.66^{a}±7.80	63.00^{ab}±5.99
60^{th} day	58.33±8.41	70.00^{b}±8.08	65.33^{ab}±12.34	65.33^{ab}±7.80
90^{th} day	58.33±17.49	72.33^{b}±6.68	72.33^{ab}±10.48	72.33^{bc}±9.84
120^{th} day	60.66±8.60	72.33^{b}±10.48	77.00^{b}±10.06	88.66^{c}±11.24
Pooled Mean	57.86±4.23	65.80±3.62	66.50±4.14	68.60±4.15

NS - Non significant

The NPN production did not show any significant variation between treatment groups. The pooled mean for NPN were found to be 57.86±4.23, 65.80±3.62, 66.50±4.14 and 68.60±4.15 mg/100 ml SRL for T_0, T_1, T_2 and T_3 groups and were comparable.

The present findings are in accordance with Jadhav (2019), Shembekar (2019) reported similar observations on cotton stalk based pelleted complete feed and Kholif and Aziz (2014) in goat fed on complete feed supplemented with cellulolytic enzymes as compared to control group. Further, Patel *et al.* (2015) also got similar findings with the average concentration of NPN (69.69±10.01) in fibrolytic exogenous enzyme @ 0.025% fed TMR group.

4.8.15 Serum total protein (g/dl) of goat in different groups

The blood samples were collected at monthly interval and serum was separated and analyzed for total protein content. The serum total protein (g/dl) values obtained in present study are presented in Table 4.20 and depicted graphically in Fig 4.17.

Table 4.20 Serum total protein (g/dl) of goats in different groups

Periods	Groups			
	T_0	T_1	T_2	T_3
0^{th} day	$6.39^{a}\pm0.13$	$6.20^{a}\pm0.09$	$6.37^{a}\pm0.08$	$6.43^{a}\pm0.08$
30^{th} day	$6.13^{a}\pm0.16$	$6.15^{a}\pm0.20$	$6.24^{a}\pm0.25$	$6.33^{a}\pm0.19$
60^{th} day	$6.25^{Aa}\pm0.35$	$6.52^{Aab}\pm0.16$	$7.03^{Bb}\pm0.24$	$6.88^{Bb}\pm0.32$
90^{th} day	$6.68^{Ab}\pm0.21$	$6.58^{Ab}\pm0.18$	$7.13^{Bb}\pm0.29$	$7.20^{Bb}\pm0.10$
120^{th} day	$6.83^{Ab}\pm0.27$	$6.65^{Ab}\pm0.22$	$6.96^{Ab}\pm0.26$	$6.95^{Ab}\pm0.15$
Pooled Mean	$6.46^{A}\pm0.11$	$6.42^{A}\pm0.08$	$6.75^{AB}\pm0.12$	$6.76^{B}\pm0.10$

$^{a,b.}$ –Means bearing different superscript in a column differ significantly (p<0.01), CD -0.37
^{A,B} - Means bearing different superscript in a row differ significantly (p<0.01), CD- 0.33

The serum total protein (g/dl) differed significantly (p<0.01) among the treatment groups and period. The total proteins on 0^{th} day were 6.39±0.13, 6.20±0.09, 6.37±0.08 and 6.43±0.08 g/dl for T_0, T_1, T_2 and T_3, respectively. The average mean value was found to be 6.46±0.11, 6.42±0.08, 6.75±0.12 and 6.76±0.10 g/dl in T_0, T_1, T_2 and T_3, respectively. The findings are in accordance with Azzaz *et al.* (2012) who reported that serum total protein was 5.8, 6.4 and 6.6 g/dl for control and treatment groups in lactating goats. This may be attributed to the improvements occurred in metabolic process as a response to the cellulases additives and experimental goat cover their protein needs. Our present findings are in line with Gado *et al.* (2007) who reported that biological treatment (cellulase, rumen liquor and *Cellumonas cellulasea*) of bagasse increases plasma total protein. Marwan *et al.* (2019) stated that total protein increased significantly (p<0.05) in enzyme supplemented group (Zymogen liquid) as compared to control feed group in buffalo calves. Mousa *et al.* (2022)[b] observed that increased (p<0.05) trends of total protein concentration in treatment groups supplemented with Calfo-Care (fibrolytic enzymes) at concentration of 0.5, 1 and 2 kg/ton of diet of DM. This effect due to improved protein digestibility in the treated groups, since Kumar *et al.* (1980) and Bush (1991) reported that serum total protein concentration reflects the nutritional grade of the animal and it has a great link with dietary protein level.

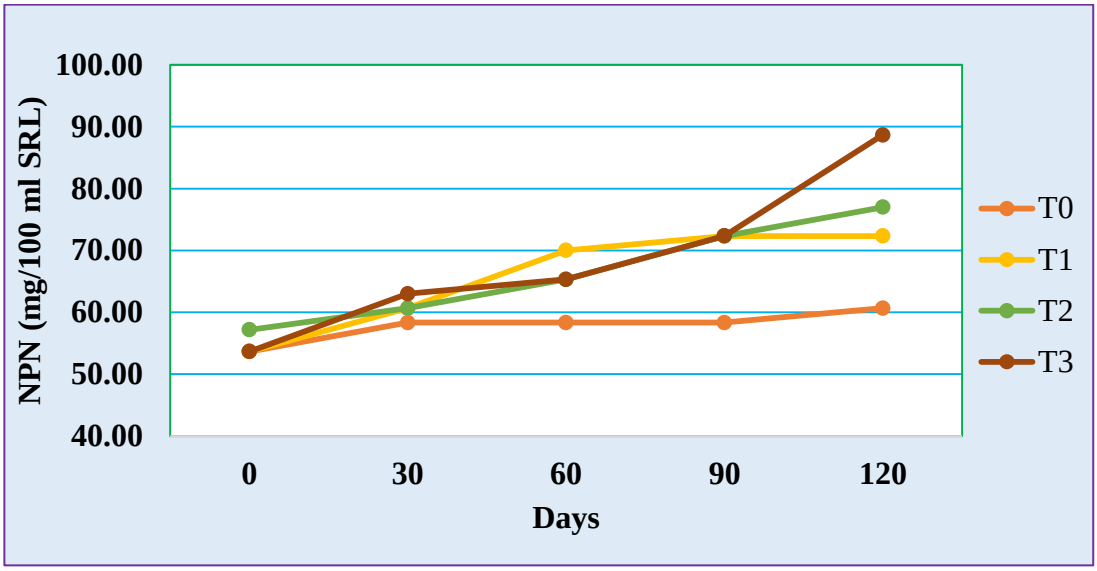


Figure 4.16 Rumen liquor NPN (mg/100 ml SRL) of goats in different groups

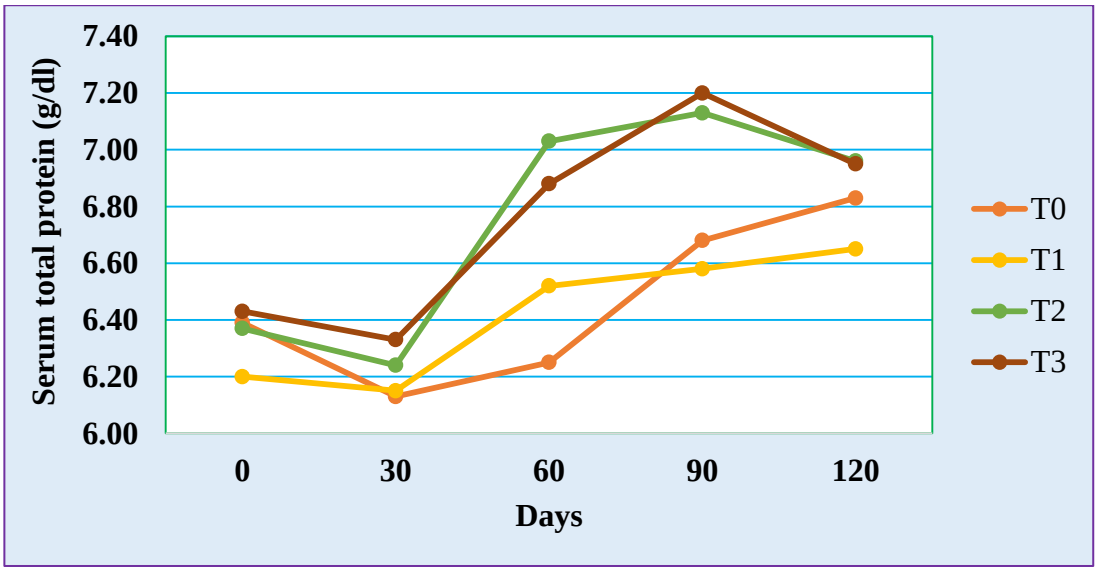


Figure 4.17 Serum total protein (g/dl) of goats in different groups

4.8.16 Serum Albumin (g/dl) of goat in different groups

The observations made with regard to serum albumin content (g/dl) of experimental goats were recorded and are presented in Table 4.21 and depicted graphically in Fig 4.18.

Table 4.21 Serum Albumin (g/dl) of goats in different groups

Periods	Groups			
	T_0	T_1	T_2	T_3
0^{th} day	2.31^{ab}±0.12	2.55±0.08	2.38^{ab}±0.04	2.52^{a}±0.16
30^{th} day	2.58^{b}±0.14	2.65±0.14	2.57^{b}±0.07	2.82^{b}±0.12
60^{th} day	2.41^{ab}±0.15	2.49±0.10	2.33^{a}±0.07	2.47^{a}±0.12
90^{th} day	2.41^{ab}±0.13	2.64±0.13	2.45^{ab}±0.07	2.53^{a}±0.11
120^{th} day	2.26^{a}±0.11	2.45±0.07	2.56^{b}±0.05	2.47^{a}±0.07
Pooled Mean	2.40±0.06	2.56±0.05	2.46±0.03	2.57±0.06

^{a,b,} –Means bearing different superscript in a column differ significantly (p<0.01), CD -0.20
NS– Non significant

The serum albumin (g/dl) were comparable among the treatment groups. The average mean value for serum albumin (g/dl) were 2.40±0.06, 2.56±0.05, 2.46±0.03 and 2.57±0.06, respectively in T_0, T_1, T_2 and T_3 whereas, there was a significant ($p<0.01$) difference at periodic intervals. The present findings are in accordance with Azzaz *et al.* (2012) who reported serum albumin as 3.2, 3.4 and 3.5 g/dl in lactating goats. Similarly, Gado *et al.* (2007) also reported that biological treatment (cellulase, rumen liquor and *Cellumonas cellulasea*) of bagasse have higher serum albumin value ($p<0.05$) which may be due higher crude protein digestibility for experimental goats.

The present findings are contrary to Kholf *et al.* (2017)[c] who revealed that blood serum albumin values were significantly higher ($p<0.05$) in basal ration fed groups of goats (Veta-Zyme Plus 15 U/Kg DM and Asperozyme 45 U/Kg DM) than goats fed without enzymes (control group). Similar findings were reported by Al-Salmany *et al.* (2019) in enzymes treated groups on eight weeks as in zero time compared with control basal ration without enzymes. Marhorus *et al.* (2019)[a,b] reported that serum albumin value numerically higher in treated olive

trees by-products with EM1 (biological product) than present findings. Marwan *et al.* (2019) stated that serum albumin increased significantly ($p<0.05$) in enzyme supplemented group (Zymogen liquid) as compared to control feed group in buffalo calves

4.8.17 Serum Globulin (g/dl) of goat in different groups

The data obtained with regard to serum globulin (g/dl) in goats from experimental groups were calculated by subtracting serum albumin from serum total protein presented in Table 4.22 and depicted graphically in Fig 4.19.

Table 4.22 Serum Globulin (g/dl) of goats in different groups

Periods	Groups			
	T_0	T_1	T_2	T_3
0th day	$4.08^{Bbc}\pm0.15$	$3.65^{Aab}\pm0.13$	$3.99^{Ba}\pm0.07$	$3.91^{ABb}\pm0.19$
30th day	$3.55^{a}\pm0.17$	$3.50^{a}\pm0.24$	$3.67^{a}\pm0.24$	$3.51^{a}\pm0.12$
60th day	$3.83^{Aab}\pm0.25$	$4.03^{Ac}\pm0.18$	$4.69^{Bb}\pm0.25$	$4.41^{Bc}\pm0.23$
90th day	$4.26^{Bcd}\pm0.16$	$3.93^{Abc}\pm0.18$	$4.67^{Cb}\pm0.28$	$4.67^{Cc}\pm0.07$
120th day	$4.56^{Bd}\pm0.21$	$4.19^{Ac}\pm0.21$	$4.40^{ABb}\pm0.24$	$4.47^{ABc}\pm0.18$
Pooled Mean	$4.06^{AB}\pm0.10$	$3.86^{A}\pm0.09$	$4.29^{B}\pm0.12$	$4.20^{B}\pm0.11$

^{a,b,c,d} –Means bearing different superscript in a column differ significantly ($p<0.01$), CD -0.34
^{A,B,C} - Means bearing different superscript in a row differ significantly ($p<0.01$), CD- 0.30

The serum globulin (g/dl) differed significantly ($p<0.01$) among the treatment groups and periods. The average mean value of serum globulin differed significantly ($p<0.01$) and observed lowest for T_1 group (3.86 ± 0.09) followed by T_0 group (4.06 ± 0.10) whereas, T_2 (4.29 ± 0.12) and T_3 (4.20 ± 0.11) groups were comparable. These results may be attributed that liquid fibrolytic enzyme treatments on cotton stalk in pelleted complete feed enhance the metabolic process as a response to increase nutrient digestibility.

The present findings are in agreement with Marwan *et al.* (2019) who stated that serum globulin increased significantly ($p<0.05$) in enzyme supplemented group (Zymogen liquid) as compared to control feed group in buffalo calves. Similarly, Azzaz *et al.* (2012) reported that serum globulin significantly ($p<0.05$) increased in lactating goats fed with basal diet containing

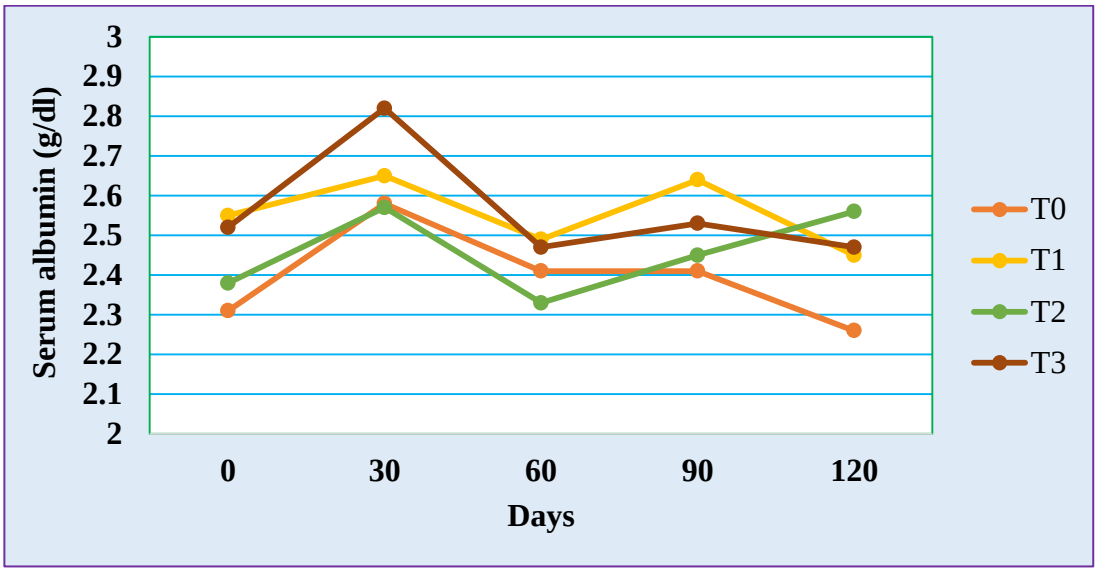


Figure 4.18 Serum Albumin (g/dl) of goats in different groups

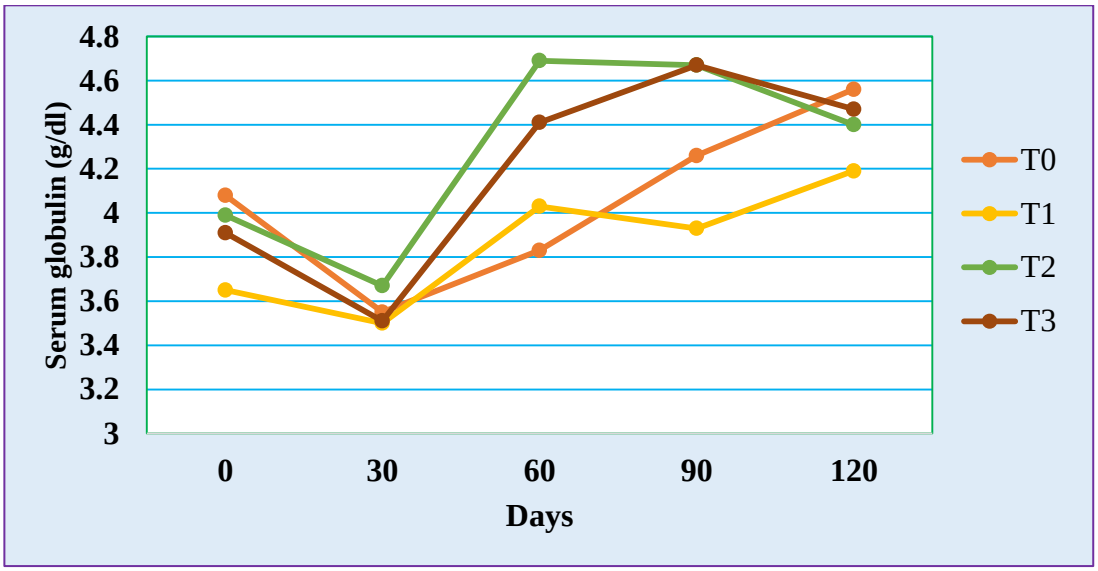


Figure 4.19 Serum Globulin (g/dl) of goats in different groups

Asperzyme @ 3.08 U/kg DM and Baciloizyme @ 1.54 U/kg when compared with control (50 % concentrate feed mixture, 25% berseem straw and 25% banana waste). This may be due to higher organic matter and crude protein digestibility when compared with control.

The present findings are contrary to Gado *et al*. (2007) who reported that biological treatment (cellulase, rumen liquor and *Cellumonascellulasea*) of bagasse have higher serum globulin value ($p<0.05$) which may be due higher crude protein digestibility for experimental goats. Kholif *et al.* (2017)[c] reported decrease in serum globulin (g/dl) in control (2.5) and enzymes (3.5) treated groups. Azzaz *et al.* (2012) who recorded serum globulin (g/dl) as 2.6, 2.9 and 3.1 in lactating goats.

4.8.18 Blood urea nitrogen (mg/dl) of goat in different groups

The observations of blood urea nitrogen (mg/dl) are presented in Table 4.23 and depicted graphically in Fig. 4.20.

Table 4.23 Blood Urea Nitrogen (mg/dl) of goats in different groups

Periods	Groups			
	T_0	T_1	T_2	T_3
0th day	$16.43^{b}\pm0.44$	$16.79^{ab}\pm1.71$	$16.84^{b}\pm1.20$	$15.38^{a}\pm1.23$
30th day	$18.27^{b}\pm2.21$	$20.47^{c}\pm1.23$	$18.03^{bc}\pm0.49$	$16.07^{ab}\pm1.26$
60th day	$14.90^{a}\pm0.64$	$20.65^{c}\pm3.05$	$21.07^{d}\pm0.77$	$17.92^{bc}\pm1.60$
90th day	$18.82^{b}\pm1.46$	$15.18^{a}\pm0.91$	$13.61^{a}\pm0.98$	$19.12^{c}\pm1.62$
120th day	$17.27^{b}\pm1.06$	$18.36^{bc}\pm0.56$	$19.43^{c}\pm0.50$	$20.33^{c}\pm0.87$
Pooled Mean	17.14±0.61	18.29±0.82	17.80±0.90	17.77±0.65

$^{a,b,c.}$ –Means bearing different superscript in a column differ significantly ($p<0.01$), CD – 2.52
NS– Non significant

The blood urea nitrogen (mg/dl) did not differ significantly amongst the treatment groups. The average mean values (mg/dl) were T_0 (17.14±0.61), T_1 (18.29±0.82), T_2 (17.80±0.90) and T_3 (17.77±0.65). The results indicated that fibrolytic enzymes treated pelleted complete feed did not affect urea level in the blood serum of growing goats.

The present findings are similar with Almaamory *et al.* (2020) who studied the effect of basal diet plus 0.5 g of exogenous enzyme Labazyme and 0.5 g exogenous enzymes Bio SB-Gold in Awassi lambs. Aboul-Fotouh *et al.* (2017) evaluated the effect of cellulolytic enzymes (Asperozyme local cellulolytic enzyme and Phyabex plus commercial cellulolytic enzyme) on serum urea concentration of lactating goats as urea is the principle end product of nitrogen metabolism in ruminants. It is synthesized in liver and extract in glomerular. The variation of plasma urea was similar with Gado *et al.* (2007) who stated that biological treatment (cellulase, rumen liquor and *Cellumonas cellulasea*) of bagasse increased plasma urea concentration.

Further, similar trends were found by Hassan and Almaamory (2016) who studied the effect of treatment of exogenous fibrolytic enzymes (EFE) on source of roughages as alfalfa hay (AH) and wheat straw (WS) with concentrate on Awassi lambs. Results showed that the serum urea nitrogen was not affected by the source of enzyme which may be due to increased efficiency of nitrogen utilization by ruminal microbes since nitrogen intake was similar in treatment.

The present findings are contrary to Mousa *et al*. (2022)[b] who recorded lesser serum urea concentration in enzymes treatment groups than control may be due to the higher protein utilization by lambs with Calfo-Care (fibrolytic enzymes), which may be related to the use of urea for protein synthesis in the hepatic pathway to compensate for decreased protein absorption.

4.8.19 Economics of feeding

In present study economics of feeding of goats was compared between all the four dietary groups by using the data generated during the experiment. The costs of ingredients were considered as per actual purchase of ingredients at farm. The cost of cotton stalk was also considered including transportation and preparation of liquid fibrolytic enzymes. The feed preparation cost of experimental feed was also considered and unit cost of feed per kg was calculated. Based on total body weight gain during the entire experimental period and feed consumed, the cost per kg unit weight gain was calculated and presented in Table 4.24.

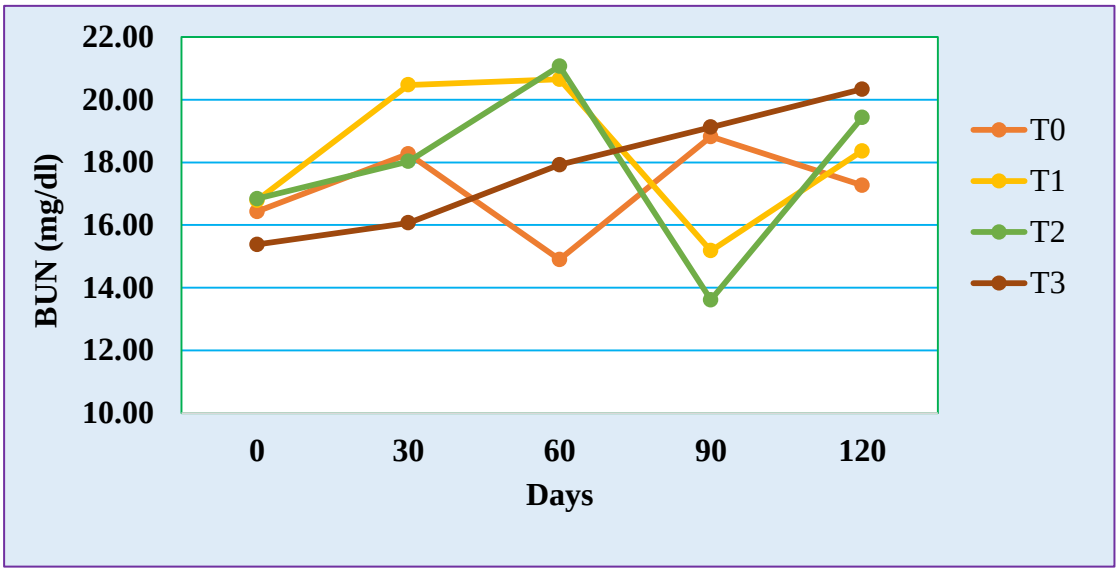


Figure 4.20 Blood Urea Nitrogen (mg/dl) of goats in different groups

Table 4.24 Economics of feeding of goats on different dietary treatments

Sr. No.	Particulars	Groups			
		T_0	T_1	T_2	T_3
1	Cost of concentrate (Rs/kg)	26.40	26.40	26.40	26.40
2	Cost of cotton stalk (Rs/kg)	1.00	1.00	1.00	1.00
3	Cost of liquid fibrolytic enzymes (Rs/Lit)	2.00	2.00	2.00	2.00
4	Cost of complete pelleted feed per kg (Rs)	11.16	11.31	11.31	11.46
5	Total feed intake per goat (kg)	88.71	86.60	89.59	89.32
6	Cost of total feed intake (Rs)	990.00	979.45	1013.26	1023.60
7	Total body wt gain per goat (kg)	8.61	8.53	8.69	9.57
8	Feed cost per kg body weight gain (Rs)	114.98	114.82	116.60	106.95

From the table it is revealed that cost of feed per kg body weight gain was lower in T_3 group as Rs (106.95) while the other treatment groups showed T_0 (114.98), T_1 (114.82) and T_2 (116.60). The T_3 group revealed Rs. 8.03 less feed cost per kg body weight gain as compared to control group. Lower feed cost and increase in total body weight gain was recorded for T_3 group due to pretreatment of cotton stalk with liquid fibrolytic enzymes showed better results as compared with other groups. Thus, without any adverse effect there was reduction in cost of feeding which has resulted maximum profit under intensive system of goat rearing.

The observations on economics of feeding are supported by Shembekar (2019) and Jadhav (2019) who reported similar findings when gram straw was replaced with cotton stalk in pelleted complete feed of goats. Kewan *et al.* (2019) also observed economic feed efficiency and higher relative economic efficiency percentage with moringa tree stalks (MS) treated yeast (*Saccharomyces cerevisiae*) (MY) and fungi (*Trichoderma reesei*) (MF) diet compared with control diet. Mahrous *et al.* (2019)[a] revealed that feed cost/kg gain and relative feed cost % were decreased as biological (CFM+ olive tree by products treated with EM_1) and chemical treatments (CFM + olive tree byproduct treated with El-mofeed contain 91% molasses, 2.5% urea and 6.5% mixing minerals and

vitamins) in tested ration as compared to control ration(CFM+ olive tree byproducts) as well as increased economic feed efficiency in biological treatment group.

SUMMARY AND CONCLUSIONS

The present experiment was undertaken to utilize cotton stalk treated with fibrolytic liquid enzymes and its incorporation in pelleted complete feed of growing goats. The experiment was conducted in three phases *viz.* extraction and assay of enzymes; evaluation of different doses and duration of treatment of enzymes and *in vivo* experiments on growing goats. The extraction and assay of enzymes, quantitative analysis and degree of degradation and thermal stability of enzymes was done in the Department of Chemical Engineering, Visvesvaraya National Institute of Technology, Nagpur. The treatment of liquid fibrolytic enzymes on shredded cotton stalk and *in vitro* digestibility of treated cotton stalk was undertaken in Department of Animal nutrition. The *in vivo* study was conducted on 24 growing goats at Instructional Livestock Farm Complex, Nagpur Veterinary College, Nagpur for 120 days with regards to growth performance, rumen fermentation, blood biochemical's and economics of feeding cotton stalk in growing goats.

Enzymes extraction and purification was done by solid state fermentation technique. Quantitative analysis of total cellulase activity was found to be 373.5 U/L and xylanase activity reported to be 1159.45 U/L. Degradation of cellulose and hemicellulose increased bioavailability of reducing sugars with increase in unfolding of complex lignocellulosic structures due to enzyme action, with overall fraction reaching up-to 94% increase in availability in a matter of 3-4 days. It was observed that the enzyme remained stable even at temperatures as high as 60°C. It was also observed that the degradation efficiency, owing to the reduced solvent content, thus concentrating the enzyme.

For standardization of dose of liquid enzymes, treatment of shredded cotton stalk with enzymes solution with different dilution (dose) and duration along with water treatment was carried out.

The pre-digestion composition and fiber fraction of enzyme treated cotton stalk revealed greater DM% and CF% of untreated cotton stalk than the various treatment groups while, NFE% was lesser in untreated cotton stalk than the treated groups. The NDF, ADF and ADL percent of untreated cotton stalk was observed to be higher than the various treatment groups whereas, the hemicelluloses and cellulose percent in various treated groups was higher except cellulose percent in treated group for 250 ml/kg and 500 ml/kg cotton stalk for 4 h found to be nearly similar to untreated cotton stalk.

The average *in vitro* dry matter digestibility (%) of untreated cotton stalk was found to be 42.10±0.41. The *in vitro* digestibility for DM of cotton stalk treated with liquid fibrolytic enzyme solution for the different treatment groups was higher than untreated cotton stalk. The *in vitro* DM digestibility in E_{250} group for 6 h (66.04 ± 0.88) and 8 h (63.62±0.92) and E_{500} group for 6 h (67.18±.05) incubation period and numerically higher in E_{500} group for 6 h incubation period due to more enzymatic activity in cotton stalk with respect to dose and duration.

The *in vitro* neutral detergent fiber digestibility (%) was significantly ($P<0.01$) higher in E_{250} group for 6 h (78.74±1.04) and E_{500} group for 6 h (80.46±1.90) and 8 h (74.80±1.65) as compared to other groups. However, numerically higher IVNDFD (%) is evident in E_{500} group for 6 h as compared to W_{2500} (64.08±0.66, 57.02±1.00, 64.46±0.90) and W_{500} (63.56±0.42, 61.80±0.46, 61.71±0.83) group for 4, 6, 8 h. The overall mean of IVNDFD was significantly higher in E_{500} (76.13±1.63) and followed by E_{250} (73.90±1.80) group.

In vitro acid detergent fibre digestibility (%) was significantly higher ($P<0.01$) in E_{250} group for 6 and 8 h (65.81±1.04, 64.76±1.52) and in E_{500} group for 6 h (66.35 ±1.79). Among these groups, numerically higher IVADFD was found in E_{500} group for 6 h as compared to E_{250} group for 6 and 8 h. The mean IVADFD values in water treatment W_{250} group for 4, 6, 8 h and 6 h were lower than E_{250} group without enzyme treatment.

The *in vivo* experiment was conducted on twenty-four growing goats, ranging between four to six months of age, randomly divided into four groups with similar body weights in each group. The goats were fed as T_0 (control) with cotton straw based pelleted complete feed and 40 percent concentrate; T_1 fed on cotton stalk based pelleted complete feed when cotton stalk was treated with fibrolytic enzymes solution 250 ml/kg for 6 h, T_2 fed on cotton stalk based pelleted complete feed when cotton stalk was treated with fibrolytic enzymes solution 250 ml/kg for 8 h and T_3 fed on cotton stalk based pelleted complete feed when cotton stalk was treated with fibrolytic enzymes solution 500 ml/kg for 6 h. All the diets were iso-nitrogenous and iso-caloric maintaining roughage to concentrate ratio as 60:40. The goats were kept in pens (stall fed system) with adequate ventilation, flooring and tying arrangements as well as feeding and watering facilities. Throughout the experimental period, all of the experimental growing goats were maintained on standard managemental practices.

The average body weights in the beginning were found to be comparable and statistically non-significant which were 15.04±1.79, 15.41±1.78, 15.49±1.45 and 15.22±2.54 kg for T_0, T_1, T_2 and T_3, respectively. At the end of experiment corresponding body weights were 19.15±0.70, 19.52±0.75, 19.70±0.61and 19.94±0.98 kg for T_0, T_1, T_2 and T_2, respectively. The average daily gains for dietary treatment groups were found to be 71.75±1.57, 71.10±2.27, 72.44±1.14 and 79.74±3.16 g/day for T_0, T_1, T_2 and T_2 group with significantly ($p<0.01$) higher in T_3 (79.74$\pm$3.16 g/day) group and statistically comparable in T_0, T_1 and T_2 fed cotton stalk based pelleted complete diet.

The average dry matter intake (DMI) was 672.76$\pm$24.54, 656.77$\pm$22.26, 671.94$\pm$20.98 and 677.37$\pm$29.70 g/day in T_0, T_1, T_2 and T_3 groups. The daily DMI from first fortnight onwards increased significantly in positive direction for each group. The average DMI percent BW for T_0, T_1, T_2 and T_3 were 4.80±0.03, 4.66±0.07, 4.66±0.03 and 4.68±0.05. The average DMI (percent BW) was comparable between groups. The fortnightly dry matter intake (percent body weights) is non-significant and comparable between the groups from first to eighth fortnight.

The average FCR for T_0, T_1, T_2, and T_3 was found to be 8.72±0.24, 8.63±0.13, 8.64±0.23and 7.83±0.30. The FCR was significantly better for T_3 group than that of T_0; however, it was comparable with T_1 and T_2.

Rumen liquor samples were collected monthly from the experimental goats and were analysed for rumen pH, rumen NH_3-N, rumen TVFA concentration, rumen total nitrogen, rumen TCA-ppt nitrogen and rumen NPN concentration of strained rumen liquor (SRL). The average values of rumen liquor pH for T_0, T_1, T_2 and T_3 was found to be 6.68±0.04, 6.69±0.03, 6.71±0.03and 6.74±0.04. The rumen liquor pH was significantly less on 0^{th} day for T_0, T_1, T_2 and T_3 and it was increased at successive month in each group. The average NH_3-N values for T_0, T_1, T_2 and T_3 were found to be 20.53±0.68, 19.64±0.71, 19.46±0.68 and 18.62±0.74mg/100 ml SRL. The rumen liquor NH_3-N varied significantly ($p<0.01$) between the periods. The average TVFA concentration was significantly ($p<0.01$) higher for T_3 (94.86$\pm$2.25) followed by T_2 (90.13$\pm$2.11), T_1 (86.46$\pm$1.36) and T_0 (84.50$\pm$2.05). The rumen liquor TVFA varied significantly ($p<0.01$) between the periods and groups. The average total nitrogen values were found to be 100.80±3.57, 109.66±3.22, 109.66±3.15and 114.33±3.80 mg/100 ml SRL for T_0, T_1, $T_2$and T_3 group. The rumen liquor total nitrogen production differed significantly ($p<0.01$) among period and groups. The average TCA-ppt-N values were found to be 42.93±2.11, 43.86±2.19, 43.16±2.27and 45.73±2.11mg/100 ml SRL forT_0, T_1, T_2 and T_3 group. The TCA-ppt-N production did not vary significantly between the groups and periodic interval. The average NPN values were found to be 57.86±4.23, 65.80±3.62, 66.50±4.14 and 68.60±4.15mg/100 ml SRL for T_0, T_1, T_2 and T_3 group. The NPN production did not show any significant variation in treatment groups.

The nutrient digestibilities of DM, OM, EE, NFE, hemicellulose, cellulose, were non-significant amongst all the dietary treatment groups. The crude protein digestibility differed significantly ($p<0.05$) and it was highest in T_2 (75.37±1.98) followed by T_3 (74.69±0.82), T_0 (control) 70.61±1.64 and lowest in T_1 (70.24±0.79). The crude fiber digestibility differed significantly ($p<0.05$) and it was highest in T_3

(47.36±3.29) followed by T_2 (43.53±4.53) and lowest and comparable (P>0.05) between in T_0 (33.97±3.13) and T_1 (35.73±3.59). The NDF and ADF digestibility was significantly (p<0.05) higher in T_2 (56.54±1.93 and 50.82±3.21) followed by T_3 (54.68±2.41 and 48.59±3.99), T_1 (50.82±1.91 and 45.13±3.87) and lower in T_0 (control) 48.28±0.87 and 43.62±1.90.

Blood samples were collected monthly from the experimental goats and were analyzed for total protein, albumin, serum globulin and blood urea nitrogen. The average total protein concentrations for T_0, T_1, T_2 and T_3 were found to be 6.46±0.11, 6.42±0.08, 6.75±0.12 and 6.76±0.10g/dl. The total protein (g/dl) differed significantly (p<0.01) amongst the treatment groups and period. The average serum albumin concentrations were found to be 2.40±0.06, 2.56±0.05, 2.46±0.03and 2.57±0.06 g/dl for T_0, T_1, T_2 and T_3 group. The serum albumin (g/dl) were comparable among the treatment groups and significantly (p<0.01) varied during periods. The serum globulin (g/dl) differed significantly (p<0.01) among the treatment groups and periods. The average serum globulin concentration was lowest for T_1 group (3.86±0.09) followed by T_0 group (4.06±0.10) whereas, T_2 (4.29±0.12) and T_3 (4.20±0.11) groups were comparable. The blood urea nitrogen values for T_0, T_1, T_2 and T_3 were found to be 17.14±0.61, 18.29±0.82, 17.80±0.90 and 17.77±0.65 mg/dl with non-significant variations. The cost of feed per kg body weight gain in T_3 group was found to be lowest (Rs. 106.95) while for other groups it was Rs. 114.98), Rs. 114.82 and Rs. 116.60 for T_0, T_1 and T_2 groups, respectively.

CONCLUSIONS

The waste fibrous crop residues such as cotton stalk can be treated with liquid firbrolytic enzyme solution containing cellulase (373.5 U/L) and xylanase (1159.45 U/L) for its enrichment.

Treatment of liquid firbrolytic enzyme solution containing cellulase (373.5 U/L) and xylanase (1159.45 U/L) @ 500 ml/kg cotton stalk for 6 h followed by 250 ml/kg cotton stalk for 6 and 8 h and its utilization in pelleted complete feed has no adverse effect on growth, rumen fermentation and blood biochemical's of goats.

It was concluded that treatment of cotton stalk with liquid fibrolytic enzyme containing cellulase (373.5 U/L) and xylanase (1159.45 U/L) @ 500 ml enzyme solution/kg cotton stalk for 6 h and its utilization in pelleted complete feed improved body weight gain with optimum rumen fermentation patterns and blood biochemical profile and also reduced cost of feed per kg body weight gain in goats under stallfed conditions. The feeding of enzyme treated cotton stalk to groups under T_3 reduced 7.5 percent cost of feed per kg body weight gain and found to be economical for goat keepers to raise goats in urban and semi urban areas. The findings will also be useful to utilize the cotton stalk a wasteful material, more effectively.

PROPOSED AREA OF FUTURE RESEARCH

The study of liquid fibrolytic enzymes treatment may also be done with other poor quality fibrous crop residues.

Liquid fibrolytic enzymes along with yeast and/or probiotics may be explored together for better combination.

The economic feasibility of supplementation of liquid fibrolytic enzymes may be explored in various system of rearing.

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APPENDICES

ANOVA for IVDMD of cotton stalk in different treatment

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal
Replications	9	247.81	27.534	-
Factor A	1	1175.628	1175.628	115.951
Factor B	1	11.041	11.041	1.089
Factor C	2	259.385	129.692	12.791
A x B	1	110.592	110.592	10.908
A x C	2	807.986	403.993	39.845
B x C	2	17.713	8.856	0.873
A x B x C	2	131.354	65.677	6.478
Error	99	1003.761	10.139	-
Total	119	3765.27	-	-

ANOVA for IVNDFD of cotton stalk in different treatment

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal
Replications	5	306.949	61.39	-
Factor A	1	3000.93	3000.93	270.189
Factor B	1	33.853	33.853	3.048
Factor C	2	35.791	17.895	1.611
A x B	1	13.546	13.546	1.22
A x C	2	496.091	248.046	22.333
B x C	2	37.031	18.516	1.667
A x B x C	2	54.243	27.122	2.442
Error	55	610.874	11.107	-
Total	71	4589.308	-	-

ANOVA for IVADFD of cotton stalk in different treatment

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal
Replications	5	65.424	13.085	-
Factor A	1	219.242	219.242	22.622
Factor B	1	2.614	2.614	0.27
Factor C	2	58.047	29.024	2.995
A x B	1	46.368	46.368	4.784
A x C	2	449.945	224.973	23.213
B x C	2	198.375	99.187	10.234
A x B x C	2	0.123	0.061	0.006
Error	55	533.044	9.692	-
Total	71	1573.183	-	-

ANOVA for fortnightly body weights of goats in different groups

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal
Replications	5	995.368	199.074	8.587
Treatments	35	1841.705	52.62	2.27
Factor A	3	17.968	5.989	0.258
Factor B	8	1819.462	227.433	9.81
A X B	24	4.276	0.178	0.008
Error	175	4057.231	23.184	-
Total	215	-	-	-

ANOVA for fortnightly body weight gain of goats in different groups

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal
Replications	5	0.859	0.172	4.037
Treatments	31	2.674	0.086	2.028
Factor A	3	0.525	0.175	4.11
Factor B	7	1.701	0.243	5.711
A X B	21	0.449	0.021	0.503
Error	155	6.594	0.043	-
Total	191	-	-	-

ANOVA for average daily gain of goats in different groups

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal
Replications	5	3816.49	763.298	4.037
Treatments	31	11886.5	383.435	2.028
Factor A	3	2331.648	777.216	4.111
Factor B	7	7559.448	1079.921	5.711
A X B	21	1995.402	95.019	0.503
Error	155	29307.42	189.08	-
Total	191	-	-	-

ANOVA for daily dry matter intake of goats in different groups

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal
Replications	5	823780.4	164756.1	8.091
Treatments	31	1492782	48154.25	2.365
Factor A	3	11545.13	3848.376	0.189
Factor B	7	1442432	206061.7	10.119
A X B	21	38804.41	1847.829	0.091
Error	155	3156435	20364.1	-
Total	191	-	-	-

ANOVA for daily dry matter intake (% BW) of goats in different groups

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal
Replications	5	1.397	0.279	2.545
Treatments	31	3.602	0.116	1.058
Factor A	3	0.672	0.224	2.039
Factor B	7	1.516	0.217	1.972
A X B	21	1.415	0.067	0.614
Error	155	17.02	0.11	-
Total	191	-	-	-

ANOVA for FCR in goats in different groups

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal
Replications	5	29.849	5.97	2.67
Treatments	31	138.542	4.469	1.999
Factor A	3	25.446	8.482	3.794
Factor B	7	83.785	11.969	5.354
A X B	21	29.311	1.396	0.624
Error	155	346.527	2.236	-
Total	191	-	-	-

ANOVA for rumen liquor pH of goats in different experimental groups

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal
Replications	5	0.088	0.018	0.982
Treatments	19	3.791	0.2	11.179
Factor A	3	0.071	0.024	1.332
Factor B	4	3.664	0.916	51.317
A X B	12	0.056	0.005	0.262
Error	95	1.696	0.018	-
Total	119	-	-	-

ANOVA for rumen liquor NH_3-N of goats in different experimental groups

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal
Replications	5	58.359	11.672	1.482
Treatments	19	986.713	51.932	6.594
Factor A	3	55.452	18.484	2.347
Factor B	4	905.945	226.486	28.758
A X B	12	25.317	2.11	0.268
Error	95	748.181	7.876	-
Total	119	-	-	-

ANOVA for rumen liquor TVFA of goats in different experimental groups

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal
Replications	5	903.842	180.768	3.218
Treatments	19	9273.158	488.061	8.689
Factor A	3	1871.092	623.697	11.104
Factor B	4	6696.367	1674.092	29.805
A X B	12	705.7	58.808	1.047
Error	95	5335.992	56.168	-
Total	119	-	-	-

ANOVA for rumen liquor total nitrogen of goats in different experimental groups

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal
Replications	5	2850.167	570.033	2.022
Treatments	19	14665.7	771.879	2.738
Factor A	3	2879.567	959.856	3.405
Factor B	4	9757.533	2439.383	8.654
A X B	12	2028.6	169.05	0.6
Error	95	26778.5	281.879	-
Total	119	-	-	-

ANOVA for rumen liquor TCA-ppt-N of goats in different experimental groups

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal
Replications	5	1292.375	258.475	1.69
Treatments	19	837.492	44.079	0.288
Factor A	3	144.958	48.319	0.316
Factor B	4	561.867	140.467	0.919
A X B	12	130.667	10.889	0.071
Error	95	14526.46	152.91	-
Total	119	-	-	-

ANOVA for rumen liquor NPN of goats in different experimental groups

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal
Replications	5	5823.242	1164.648	2.533
Treatments	19	9497.425	499.864	1.087
Factor A	3	1990.625	663.542	1.443
Factor B	4	5661.133	1415.283	3.078
A X B	12	1845.667	153.806	0.335
Error	95	43674.93	459.736	-
Total	119	-	-	-

ANOVA for nutrient digestibility in experimental goats

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal
Dry Matter				
Treatments	3	82.095	27.365	1.226
Error	12	267.811	22.318	-
Total	15	-	-	-
Organic Matter				
Treatments	3	100.293	33.431	1.419
Error	12	282.705	23.559	-
Total	15	-	-	-
Crude Protein				
Treatments	3	89.979	29.66	3.617
Error	12	98.077	8.323	-
Total	15	-	-	-
Ether Extract				
Treatments	3	189.674	63.225	1.215
Error	12	624.308	52.026	-
Total	15	-	-	-
Crude Fibre				
Treatments	3	85.979	28.66	3.617
Error	12	95.077	7.923	-
Total	15	-	-	-
Nitrogen free extract				
Treatments	3	165.035	55.012	1.548
Error	12	426.572	35.548	-
Total	15	-	-	-
Neutral Detergent Fibre				
Treatments	3	166.585	55.528	4.683
Error	12	142.303	11.859	-
Total	15	-	-	-
Acid Detergent Fibre				
Treatments	3	168.585	56.528	4.683
Error	12	145.303	13.859	-
Total	15	-	-	-
Hemicellulose				
Treatments	3	80.777	26.926	0.187
Error	12	1728.572	144.048	-
Total	15	-	-	-
Cellulose				
Treatments	3	146.395	48.798	1.25
Error	12	468.626	39.052	-
Total	15	-	-	-

ANOVA for total protein of goats in different experimental groups

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal
Replications	5	3.99	0.798	3.335
Treatments	19	13.469	0.709	2.963
Factor A	3	3.008	1.003	4.191
Factor B	4	8.752	2.188	9.146
A X B	12	1.708	0.142	0.595
Error	95	22.728	0.239	-
Total	119	-	-	-

ANOVA for albumin of goats in different experimental groups

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal
Replications	5	0.297	0.059	0.835
Treatments	19	1.933	0.102	1.431
Factor A	3	0.572	0.191	2.684
Factor B	4	0.904	0.226	3.179
A X B	12	0.457	0.038	0.535
Error	95	6.752	0.071	-
Total	119	-	-	-

ANOVA for globulin of goats in different experimental groups

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal
Replications	5	3.36	0.672	3.259
Treatments	19	18.778	0.988	4.792
Factor A	3	3.035	1.012	4.905
Factor B	4	12.686	3.172	15.378
A X B	12	3.057	0.255	1.235
Error	95	19.592	0.206	-
Total	119	-	-	-

ANOVA for blood urea nitrogen of goats in different experimental groups

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal
Replications	5	41.099	8.22	0.743
Treatments	19	949.632	49.981	4.515
Factor A	3	55.979	18.66	1.686
Factor B	4	233.897	58.474	5.282
A X B	12	659.755	54.98	4.967
Error	95	1051.643	11.07	-
Total	119	-	-	-

VITA

The author Dr. Suresh Fattuji Nipane was born on 12th December, 1976 at Sakoli, Taluka Sakoli, Dist Bhandara, Maharashtra. Author after completion of secondary education and higher secondary education moved to Nagpur Veterinary College, Nagpur for pursuit of his graduation in 1997 and finished his B. V. Sc. & A.H. degree in September, 2002. Also completed M. V. Sc. degree in Animal Nutrition discipline from Nagpur Veterinary College, Nagpur in December, 2004. Author joined as a Technical Advisor in Contract Broiler Farming in A to Z Poultry Services in Kalyan, Then Technical Manager in Broiler Breeder Farm at Suman Hatchery Pvt. Ltd., Raipur (C.G.) and then at Phenix Poultry, Parihat, Jabalpur (M.P.) as a Nutritionist. Then author get selected as a Livestock Development Officer through MPSC and joined in Veterinary Dispensary, Grade I, Parwa, Tah. Ghatanji, Dist. Yeotmal in year December, 2007. During Government service, author made active involvement various activities like Vaccination, Artificial Insemination, Work camp, Infertility camp, Cattle Exhibition, Calf Rally, various training programmes taluka

Author joined in Taluka Veterinary Mini Polyclinic, Mohadi, Dist. Bhandara in year June, 2010 after regular transfer. Author motivated farmers for quail farming and also introduced Kadaknath breed of poultry in taluka for rearing and income generation through Central Poultry Development Organization, Goregaon, Mumbai. Also, author was involved in various activity under ATMA like preparation of Silage, Azzola cultivation, Urea treatment and trainings to Pashu Sakhi. Author participated in "EKATMIK PANLOT VYATHAPAN KARYKRAM JANJAGRUTI ABHIYAN" under Taluka Agriculture Officer.

After, author transferred to "Animal Check Post" Sakoli, Dsit. Bhandara in August, 2015 and worked in Rinderpest eradication programme under the guideline of Central and Maharashtra Government. Simultaneously, author worked in various beneficiary scheme of state and tribal while working in District Deputy Commissioner Office, Bhandara. Author awarded have been Best Implement of Navinyapurna Yojana" for the year 2015-16 and 2016-17.

It was author's desire to become a PhD Scholar. Hence author joined the Department of Animal Nutrition, Nagpur Veterinary College, Nagpur in February, 2020 for Doctor of Philosophy degree through Department of Animal Husbandry, Government of Maharashtra as a in-service candidate. During the studies, he worked in sponsored project entitled "Scale-Up and techno economic feasibility studies on complete feed for animals using ozonated cotton stalk as roughage" at department of animal nutrition, NVC, Nagpur; and Author considers himself fortunate enough to serve the voiceless animals and this noble profession. Author awarded "**GUNWANT PASHUVAIDAKIY PURASKAR**" for year 2014-15 on 20th May, 2022 on the occasion of "Pushusawardhan Din" and "**Sri Ram Singh Memorial Animal Husbandry Award – 2023".** The dedication and sincere efforts performed during three years resulted in the submission of the present thesis successfully.

THESIS ABSTRACT

a)	Title of thesis	:	**EVALUATION OF FIBER DEGRADING ENZYMES IN COTTON STALK BASED PELLETED COMPLETE FEED OF GROWING GOATS**
b)	Full Name of Student	:	**NIPANE SURESH FATTUJI**
c)	Name & Address of Advisor/ Guide	:	**Dr. S. B. KAWITKAR** Professor and Head Department of Animal Nutrition Nagpur Veterinary College, College
d)	Degree to be Awarded	:	**DOCTOR OF PHILOSOPHY**
e)	Year of award of Degree	:	**2023**
f)	Major subject	:	**ANIMAL NUTRITION**
g)	Total number of pages in the thesis	:	**140**
h)	Number of words in the thesis abstract	:	**638**
i)	Signature of student	:	
j)	Signature, Name & Address of forwarding authority	:	**Associate Dean** **Nagpur Veterinary College** **Nagpur**

ABSTRACT

The experiment was conducted to utilize cotton stalk treated with fibrolytic liquid enzymes and its incorporation in pelleted complete feed of growing goats as a sole roughage source. The enzymes extraction and purification

was done by solid state fermentation technique. As per quantitative analysis total cellulase activity was found to be 373.5 U/L and xylanase activity was found to be 1159.45 U/L. The enzyme was stable even at temperatures as high as 60°C

Standardization of dose of liquid enzymes, treatment of shredded cotton with enzymes solution was done with different volume per kg cotton stalk and incubation periods. The pre-digestion composition and fibre fraction of enzyme treated cotton stalk revealed greater DM% and CF% of untreated cotton stalk than the various treatment groups. The NDF, ADF and ADL percent of untreated cotton stalk was also higher than the various treatment groups.

The average *in vitro* dry matter digestibility (%) of untreated cotton stalk was found to be 42.10±0.41. The *in vitro* digestibility for DM, NDF and ADF of cotton stalk treated with liquid fibrolytic enzyme solution for the different treatment groups was significantly higher ($p<0.01$) in E_{250} group for 6 h and 8 h incubation and in E_{500} group for 6 h incubation.

The *in vivo* experiment was conducted on twenty-four growing goats for 120 days and fed as T_0 (control) fed cotton straw based pelleted complete feed and 40 percent concentrate; T_1 fed treated fibrolytic enzymes solution 250ml/kg for 6 h cotton stalk based pelleted complete feed, T_2 fed treated fibrolytic enzymes solution 250ml/kg for 8 h cotton stalk based pelleted complete feed, and T_3 fed treated fibrolytic enzymes solution 500ml/kg for 6 h cotton stalk based pelleted complete feed. The roughage to concentrate ratio was maintained as 60:40 in each treatment. The average body weights in the beginning were found to be comparable and statistically non-significant for all the groups. The corresponding body weights at the end of experiment also revealed non-significant variations. The average daily gains for dietary treatment groups were found to be 71.75±1.57, 71.10±2.27, 72.44±1.14 and 79.74±3.16g/day for T_0, T_1, T_2 and T_3 group, respectively with significantly ($p<0.01$) higher ADG in T_3 (79.74±3.16 g/day) group. The average dry matter intake (DMI) was 672.76±24.54, 656.77±22.26, 671.94±20.98 and 677.37±29.70 g/day in T_0, T_1, T_2 and T_3 groups. The average DMI percent BW for T_0, T_1, T_2 and T_3 were 4.80±0.03, 4.66±0.07, 4.66±0.03 and

4.68±0.05. The FCR was significantly better for T_3 group than that of T_0; however, it was comparable with T_1 and T_2.

The rumen fermentation pattern determined monthly revealed non-significant variations for pH, however significantly lower NH_3-N and higher TVFA concentrations and total nitrogen was recorded for enzyme treatment groups. However, rumen TCA-ppt-N and NPN did not alter due to enzyme treatment of cotton stalk.

The nutrient digestibilities of DM, OM, EE, NFE, hemicellulose, cellulose, were non-significant amongst all the dietary treatment groups. The crude protein digestibility was significantly ($p<0.05$) higher in T_2 (75.37±1.98) and crude fibre digestibility was significantly ($p<0.05$) higher in T_3 (47.36±3.29) groups. The NDF and ADF digestibility was significantly ($p<0.05$) higher in T_2 (56.54± 1.93 and 50.82±3.21).

The serum total protein and globulin (g/dl) differed significantly ($P<0.01$) amongst the treatment groups and period, however serum albumin and blood urea nitrogen did not alter significantly. The cost of feed per kg body weight gain in T_3 group was lowest as Rs. 106.95 and it was less by Rs. 8.03 as compared to control group.

It was concluded that treatment of cotton stalk with liquid fibrolytic enzyme containing cellulase (373.5 U/L) and xylanase (1159.45 U/L) @ 500 ml enzyme solution/kg cotton stalk for 6 h and its utilization in pelleted complete feed improved body weight gain with optimum rumen fermentation patterns and blood biochemical profile and also reduced cost of feed per kg body weight gain in goats under stallfed conditions. The feeding of enzyme treated cotton stalk to growing goats under T_3 was 7.5 percent more economical than control group (T_0) in terms of body weight gain.

011प्रबंध सारांश

अ.	प्रबंधाचे शिर्षक	:	**"वाढीव शेळ्यांमध्ये कापसाच्या पराठीचा संपुर्ण कांडीखाद्यातील तंतुमय पदार्थांचे विघटन करणारे विकराचे मुल्यांकन"**
ब.	विद्यार्थ्याचे पुर्ण नाव	:	**सुरेश फत्तुजी निपाने**
क.	मार्गदर्शकाचे नाव आणि पत्ता	:	**डॉ. एस. बी. कविटकर प्राध्यापक व विभाग प्रमुख पशुपोषण आहारशास्त्र विभाग, नागपूर पशुवैद्यक महाविद्यालय,नागपूर**
ड.	प्रदान करण्यात येणारी पदवी	:	**आचार्य पदवी (पीएच. डी.)**
इ.	पदवी प्रदान करण्याचे वर्ष	:	**२०२३**
फ.	मुख्य विषय	:	**पशुपोषण आहारशास्त्र**
ग.	प्रबंधातील एकुण पृष्ठे	:	१४०
ह.	सारांषातील एकुण शब्द	:	६२७
ई.	विद्यार्थ्याची स्वाक्षरी	:	
ज.	अग्रेषित करणाऱ्याची स्वाक्षरी, नाव आणि पत्ता	:	

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सारांश

सदर संशोधन हा तंतुमय पदार्थाचे विघटन करणाऱ्या द्रवरूप विक्राची प्रक्रिया करण्यात आलेल्या कापसाच्या पराठ्याच्या वापर करण्यासाठी आणि त्यांच्या संपूर्ण कांडीखाद्य वापर करून वाढत्या शेळ्यासाठी एकमेव उताराच्या स्त्रोत म्हणून करणे असा

होता. विकराचे अर्क व शुद्धीकरण हे घन स्थितीतील आंबवणाच्या प्रक्रियेद्वारे करण्यात आले. सांख्यिकी दृष्ट्या संपूर्ण सेल्युलेज क्रियाकलप ही ७३७.५ युनिट/ लिटर इतकी आढळून आली व झायलनेज क्रियाकलप ही ११५९.४५ युनिट/ लिटर इतकी आढळली. सदर विकरे हे ६० डिग्री अंश सेल्सिअस तापमानाला स्थिर आढळून आले.

द्रावण विकराच्या प्रमाणाचे मानकीकरण हे द्रवरूप विकरे विविध प्रमाणात प्रति एक किलो बारीक तुकडे केलेल्या पराठ्यासोबत वेगवेगळ्या वेळासाठी उष्मायन करून ठरवण्यात आले. विकरे उपचारीत पराठेची पूर्व पचन रचना आणि फायबर अंशाने उपचार न केलेल्या पराठीच्या विविध उपचार गटापेक्षा जास्त शुष्क (टक्के) आणि कच्चे तंतुमय (टक्के) पदार्थ दिसून आले. उपचार न केलेल्या पराठीत एनडीए, एडीएफ आणि एडिएल टक्के देखील विविध उपचार गटापेक्षा जास्त होते.

उपचार न केलेल्या पराठीची इनविट्रो कोरड्या पदार्थाची पचनक्षमता (टक्के) सरासरी ४२.१०±०.४१ असल्या चे आढळून आले. वेगवेगळ्या उपचार गटासाठी तंतुमय पदार्थाचे विघटन करणाऱ्या द्रवरूपी विकराने उपचार केलेल्या पराठ्याच्या शुष्क पदार्थ, एनडीए आणि पीडीएफ साठी इन विट्रो पचन क्षमता इ$_{२५०}$ गटामध्ये ६ तास आणि ८ तास उष्मायनासाठी आणि इ$_{५००}$ गटामध्ये ६ तास उष्मायनासाठी लक्षणीयरीत्या जास्त ($p<0.01$) होती.

इन विवो प्रयोग १२० दिवसांसाठी २४ वाढणाऱ्या शेळ्या वर करण्यात आला आणि टी$_{०}$ (नियंत्रण) पराठीवर आधारित संपूर्ण कांडी खाद्य आणि ४०% खुराक म्हणून खायला देण्यात आले. टी$_{१}$ गटाला पराठीवर सहा तासाकरिता तंतुमय पदार्थाचे विघटन करणारे विकराची प्रक्रिया केलेले संपूर्ण खाद्य देण्यात आले, टी$_{२}$ गटाला पराटीवर ८ तासाकरिता तंतुमय पदार्थाचे विघटन करणारे विकराची प्रक्रिया केलेले संपूर्ण कांडीखाद्य देण्यात आले आणि टी$_{३}$ गटाला पराठीवर ६ तास करिता तंतुमय पदार्थाचे विघटन करणारे विकराची प्रक्रिया केलेले संपूर्ण कांडीखाद्य देण्यात आले. प्रत्येक गटातील कुटार व खुराकाचे ६०:४० चे प्रमाण ठेवण्यात आले. सुरुवातीला शरीराचे सरासरी वजन सर्व गटासाठी तुलनात्मक आणि शांख्यिकीय दृष्ट्या गैर महत्वपूर्ण असल्याचे आढळले. प्रयोगाच्या शेवटी शरीराच्या संबंधित वजनामध्येही लक्षणीय फरक दिसून आला. आहारातील उपचार गटासाठी सरासरी दैनंदिन वजन ७१.७५±१.५७, ७१.१०±२.२७, ७२.४४±१.४४ आणि ७९.७४±

३.१६ ग्राम/ दिवस हे टी०, टी१, टी२ आणि टी३ अनुक्रमे गटांमध्ये आढळून आले व सोबत लक्षणीय ($p<0.01$) जास्त सरासरी टी3 गटामध्ये (७९.७४±३.१६ ग्राम/ दिवस) आढळून आले. टी०, टी१, टी२ आणि टी३ गटांमध्ये सरासरी दैनंदिन कोरड्या पदार्थाचे सेवन (डी एम आय) ६७२.७६±२४.५४, ६५६.७७±२२.२६, ६७१.९४±२०.९८ आणि ६६६.३७±२९.७० ग्रॅम/ दिवस होते. टी०, टी१, टी२ आणि टी३ गटासाठी सरासरी दैनंदिन कोरड्या पदार्थाचे सेवन वजनाच्या टक्केवारी मध्ये ४.८०±०.०३, ४.६६±०.०७, ४.६६±०.०३ आणि ४.६८±०.०५ होते. टी० (नियंत्रण) गटापेक्षा टी३ गटासाठी एफ सी आर लक्षणीय रित्या चांगला होता, तथापि ते टी१ आणि टी२ गटाशी तुलना करता येण्यासारखे होते.

रूमंथिका किण्वन प्रक्रिया माध्यमात महिनेवारीत सामू मध्ये गैर महत्वपूर्ण फरक दिसून आला तथापि लक्षणीय पणे कमी अमोनिया नायट्रोजन आणि ओलाटाइल फॅटी ऍसिड साद्रता आणि एकूण नायट्रोजन, विकरे उपचार केलेल्या गटासाठी नोंदविले गेले. तथापि रूमंथिकेत टीसीए-पीपीटी-नायट्रोजन आणि एनपीएन पराठीवरील विक्राच्या उपचारामुळे बदललेले नाहीत.

ड्राय मॅटर, ऑरगॅनिक मॅटर, इथर एक्सट्रॅक्ट, नायट्रोजन फ्री एक्स्ट्रॅक्ट, हेमीसेलूलोज, सेलूलोज ची पोषक पचनक्षमता सर्व आहारातील उपचार गटामध्ये लक्षणीय नव्हती. कच्चे प्रथिनाची (क्रूड प्रोटीन) पचनक्षमता टी२ गटामध्ये (७५.३७±१.९८) लक्षणीय ($p<0.05$) जास्त होती आणि टी3 गटामध्ये (४७.३६±३.२९) कच्चे तंतुमय पदार्थ (फूड फायबर) पचनक्षमता लक्षणीय ($p<0.05$) जास्त होती. टी२ गटामध्ये (५६.५४±१.९३ आणि ५०.८२±३.२१) मध्ये एनडीए आणि एडीएफ पचनक्षमता लक्षणीयरीत्या ($p<0.05$) जास्त होती.

रक्तद्रवातील एकूण प्रथिने व ग्लोबोलीन (ग्राम/ डीएल) उपचार गट आणि कालावधी दरम्यान लक्षणीय भिन्न ($p<0.01$) तथापि रक्तद्रव्य अल्बुमिन आणि रक्त युरिया नायट्रोजन लक्षणीय बदललेले नाहीत. टी3 गटातील प्रति किलो शरीराचे वजन वाढण्यासाठी कांडी खाद्याची किंमत सर्वात कमी रुपये १०६.९५ आणि तो टी० (नियंत्रण) गटाच्या तुलनेत रुपये ८.०३ ने कमी झाला.

यावरून असा निष्कर्ष काढण्यात आले की, पराठेवर सेलुलेज (३७३.५ युनिट/लिटर) आणि झायलनेज (११५९.४५ युनिट/ लिटर) ५०० मिली विकर द्रावण/ किलो

पराठेवर तंतुमय पदार्थाचे विघटन करणाऱ्या द्रवरूपी विकरासह उपचार केल्याने 6 सहा तासासाठी आणि संपूर्ण कांडी खाद्याच्या उपयोगामुळे शरीर वजनात व सोबतच रोमन थिकेत किण्वन प्रक्रियेत आणि रक्त जैव रासायनिक माध्यमांमध्ये सुधारणा आढळली आणि तसेच बंदिस्त पद्धतीतील शेळ्यामध्ये संपूर्ण कांडी खाद्यावर प्रति किलो वजन वाढण्यात किंमत कमी करता आली.