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Optimization of feeding levels of ground nut cake as supplement to the wheat straw based ration by Sani method of feeding in steers

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Abstract
Five steers (195.0 kg live weight) and age were individually housed and fed a basal diet of wheat straw *ad libitum*. The dose response effect of GNC on straw intake and nutrient utilization were tested in a 5x5 latin square design, with five experimental periods of 30 days each. The total intake capacity of each animal during the various experiments was known by feeding them the ration in the form of a 'Sani'. The *in vivo* experiments were correlated with *in vitro* Hohenheim gas technique. The supplementation of GNC to wheat straw based diet was found to exert significant (p<0.05) effect on total intake of dry matter (DMI) and organic matter (OMI) by steers, N-retention significantly (p<0.05) but was lower as compared to its comparable values recorded in steers when GNC was given from 10 to 30% level. It was concluded that performance of the steers improved statistically at 5% level. Though feeding additional levels of groundnut cake could improve the performance but they were non significant.

Keywords: Ground nut cake, Sani, *in vitro* gas production, purine derivatives

Introduction
The farmers in rural areas have vogue of feeding the crop residues which is mostly cereal straws to the cattle along with undefined quantity of the grains, agro-industrial products such as brans, oil cakes, concentrates etc. But this quantity is not scientifically defined and varies every often as farmers do not know how much of the single ingredient to be fed. Quantification of this supplementation would help the farmers to feed the animals to maximize production, reap the benefits and also to improve the economy.
The present study also aimed at the correlation between *in vitro* and *in vivo* methods of estimation so that the costly animal experimentation can be replaced by *in vivo* methods of estimation.
Ground nut cake is commonly used single ingredient in the rural areas in places where farmer grows ground nuts in their own fields or a person possessing oil mills or a progressive farmer. Strategic supplementation for increased straw intake and utilization with oilseed cakes (Low fibre-high protein feeds which are potential Nitrogen sources). The selected supplements will not reduce intake and utilization of the basal diet but instead have the potential for enhancing them (McMeniman *et al*., 1988; Sampath *et al*., 1995; Dutta *et al*., 1999) [10, 17, 7]. When non-protein nitrogen and true protein sources were compared as supplements, generally the protein supplements were superior to non-protein nitrogen for rumen digestion of dry matter, organic and cell wall components (Kropp *et al*., 1977) [9]. It has been suggested that the presence of amino acids, peptides or proteins is more important for cellulolytic microorganisms than for those utilizing starch as a substrate (Huque and Thomsen, 1984) [8]. However, variation in the proportion of straw in the diet will affect the size of the response to supplementary nitrogen (Oldham and Smith, 1982) [15].

Materials and Methods
Phase I: *In vivo* Experiment in steers
Five intact steers (195.0± 6.4 kg live weight) were individually housed and fed a basal diet of wheat straw *ad libitum*. The dose response effect of GNC on straw intake and nutrient utilization were tested in a 5x5 latin square design, with five experimental periods of 30 days, in which the first 21 days were the preliminary period, 9 day in metabolic stalls, having arrangement to collect urine and faeces separately, for 6 days collection of feces and urine and the last day for collection of blood samples.

Each steer (G1, G2, G3, G4 or G5) was allocated to one of the five levels of GNC *viz*. 0 (GNC_0), 5 (GNC_5), 10(GNC_{10}), 20(GNC_{20}) and 30 (GNC_{30}) parts of straw as follows:
Body weights were recorded (before feeding and watering) at three instances during each switchover trial, *viz*., at the beginning of the feeding trial and at the start and at the end of metabolic trial. A mixture of mineral mixture and salt was prepared in 75:25 proportions and around 50 g of this mixture was added daily in the form of a 'Sani' (product of water soaked supplement and wheat straw after mixing). To assess the balance of nitrogen, microbial nitrogen supply, plane of nutrition, digestibility of nutrients and supplementary effect on straw utilization, five metabolic trials were conducted during each experiment, i.e., total twenty digestion cum metabolic trials were executed.
Sampling of feed, faeces, urine, residual feeds were done according to standard protocols and analyzed for proximate and Van soest principles. Purine derivatives and Creatinine contents of urine were analyzed by HPLC method as per Resines *et al* (1992) [16].
The *in vitro* incubation of the potential supplements used earlier in phase I were carried out to estimate feed value of complete diet based on wheat straw. The objective of the work was to simulate various supplementation experiments *in vitro*, by incubating a pure roughage (wheat straw), a pure supplement of GNC and mixtures of the two, with increasing inclusion levels of the supplement, in a short term batch incubation system (Hohenheim gas test) to investigate the extent to which such methods can replace actual animal feeding trials.

Phase-II: Prediction of optimal level of inclusion of some commonly used supplements *in vitro* to estimate feed value of complete diet and relationship with *in vivo* feeding trials for true supplementary effect
In vitro incubations were carried out in the Hohenheim gas test system according to Menke *et al* (1979) [11], as modified by Blummel *et al*. (1997) [2]. The rumen fluid was obtained from two fistulated buffaloes fed a mixture of wheat straw and concentrate at maintenance level. Wheat straw and the target supplements *viz*., GNC, WG, WB and BCM were incubated as sole substrates and at 5 supplementation levels of wheat straw-target supplement. The protocol as described in table 1 was applied to three independent experiments, which were conducted during this phase to determine kinetics of gas production, truly degradable organic matter in the rumen (TDOMR)-microbial biomass production (MBP)-partitioning factor (PF) and TVFA-enzyme profile, respectively.

Table 1: The protocol as described in table was applied to three independent experiments

Supplement	Levels of WS: Supplement	Total amount of substrate	No of sets	No of replicates
Groundnut cake	Pure WS, 95:05, 90:10, 80:20, 70:30, 60: 40, Pure GNC	200 mg	2 for each source	3 syringes

Measurement of *in vitro* gas production kinetics
The preliminary screening of various supplement levels was done by *in vitro* gas production technique (Menke *et al*., 1979; Menke and Steingass, 1988) [12, 11]. The rate and extent of gas production were estimated by fitting modified Mitscherlich equation of Orskov and McDonald model of single exponential equation with lag phase to study gas production kinetics of substrate containing wheat straw, various ratios of wheat straw: supplement and supplement alone. The fermentation kinetics was estimated by means of nonlinear regression as follows.: $Y= b \times [1-e^{-c\,(t-L)}]$
The substrate specific times were defined by the half time ($T_{1/2}$) of asymptotic gas production and calculated as (Blummel, 2000) [7]: $T_{1/2} = \ln 2/c$
Voluntary feed intake potential (g DM kg^{-1} BW $^{0.75}$) was calculated from *in vitro* gas production kinetics parameters using the equations given by Blummel and Becker (1997) [1] as follows:

Intake (g DM kg^{-1} BW $^{0.75}$) = 18.90 + 0.23(a+b) + 687xc+0.11x CP
Organic matter weighed into the syringe = {200 x DM in percentage/100} – {200 x (DM in percentage/100)x(Ash in percentage/100)}. This value was termed as 'c'.
Percent organic matter degradability = (a-b)*100/c. i.e.,
TDOM = Feed (OM) incubated - residue (OM)
The partitioning factor (PF) was calculated according to Blummel *et al* (1997) [1] as
PF = (mg truly degraded organic matter)/ml gas produced.

The estimation of microbial biomass production for a given incubation was made by using TDOMR and gas production in the following way (Blummel and Lebzien, 2001) [5]:

MBP = TDOMR– (net gas volume*SF)

Where, SF represents stoichiometrical factor, which has a constant value 2.2 for most of the incubations (Blummel and Fernandez-Rivera, 2002) [6]. Enzymatic activity such as CM Case (CM Case, EC 3.2.1.4) activity was estimated in samples according to the procedure described by Miller (1959) [13]. All the data were statistical analyzed as 5x5 switch over design as per the procedure given by Snedecor and Cochran (1994) [18].

Results and Discussion
The chemical composition of the various feed ingredients was comparable and statistically insignificant between treatments (table 2).

Table 2: Chemical composition of feeds (% DM)

Parameters	OM	CP	TA	NDF	ADF
Wheat straw	93.52	3.6	6.47	81.27	54.2
Groundnut cake	93.59	44.97	6.41	32.35	19.33

The supplementation of GNC to wheat straw based diet was found to exert significant ($p<0.05$) effect on total intake of dry matter (DMI) (fig 1) and organic matter (OMI) by steers when expressed as g/d or per kg metabolic body size (g/kg $W^{0.75}$) or % live weight (%LW) and digestibility coefficient of DM and OM at 5% level ($p<0.05$). However, no additional beneficial effect was evident when its level was increased beyond 10% (GNC-10) in the diet. The crude protein digestibility was improved substantially in animals given GNC-30 followed by comparable values observed among steers given GNC-20 or GNC-10. Net balance of N (g/d) improved linearly ($p<0.05$) in animals as dietary level of

GNC increased from GNC-5 to GNC-30, although animals of control group (GNC-0) were in negative nitrogen balance.

In the *in vitro* model, gas produced in the gas technique is the direct gas produced (fig 2) as a result of fermentation (CO_2 and CH_4) and the indirect gas produced from the buffering of SCFA. In case of straw fermentation, about 53% of the gas volumes consisted of CO_2 released from the bicarbonate buffer produced upon buffering the SCFA (Blümmel *et al.*, 1999) [3] and the rest is evolved directly from fermentation. It has been demonstrated that *in vitro* gas measurements reflect only SCFA production and that an inverse relationship can exist between gas volume (or SCFAs) and microbial biomass production (Naga and Harmeyer, 1975) [14] particularly when both were related to a unit of substrate truly degraded (Blümmel, 2000) [4]. A significant negative relationship (R^2 = - 0.83**) between $T_{1/2}$ of substrates and their relative intake as digestible organic matter (DOMI, g/kg $W^{0.75}$) in steers was recorded which indicates that substrates having relatively lower half time of asymptotic gas production may likely to have higher DOMI by the animals which will ultimately lead to higher animal productivity. A strong positive correlation between OMD (%) *in vivo* and *in vitro* TDOMR (%) was evident (R^2 =0.89) for the range of feeds used during this experiment irrespective of dietary treatments. Similarly, TDOMR *in vitro* was found to be strongly correlated (R^2 =0.92) with DOMI (g/kg $W^{0.75}$) of steers and to half time. Application of these shortcuts can be very helpful in screening of large number of samples from the field or crop improvement programs where availability of feeds for longer term experimentation may meet logistical problems (Zerbini and Thomas, 1999) [19].

The excretion of allantoin and uric acid in urine by steers increased linearly (p<0.05) with increase in proportion of dietary GNC. Similarly, urinary excretion of total PD (mmol/d) increased significantly (p<0.05) in animals as a consequence of supplementation of GNC at graded levels by steers. However, the proportional contribution of allantoin to uric acid in total PD remained comparable without any significant difference irrespective of dietary levels of GNC. However, efficiency of microbial nitrogen synthesis, when expressed on the basis of GNC per kg degradable organic matter in rumen (g N kg-1 DOMR), found to be significantly improved up to 30% level of GNC supplementation (GNC-30), although no significant difference (P>0.05) was evident between animals on treatments GNC-5 & GNC-10 and GNC-20 & GNC-30.

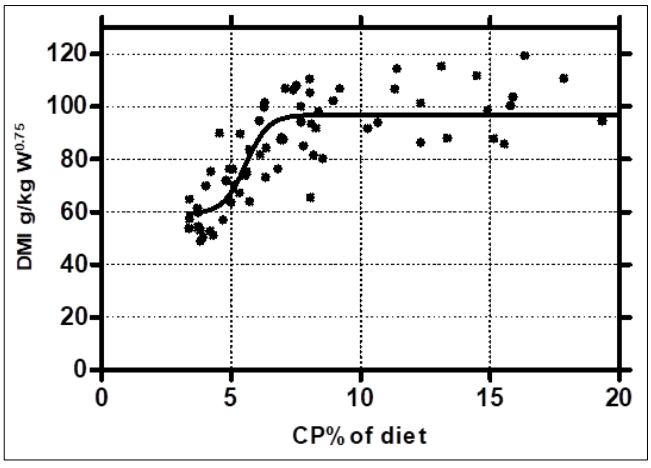

Fig 1: Plot of total DMI (g/kg $W^{0.75}$) as a response to CP content (%DM) of the diets (Total DMI =CP% x 2.45 + 63.99, R= 0.61**)

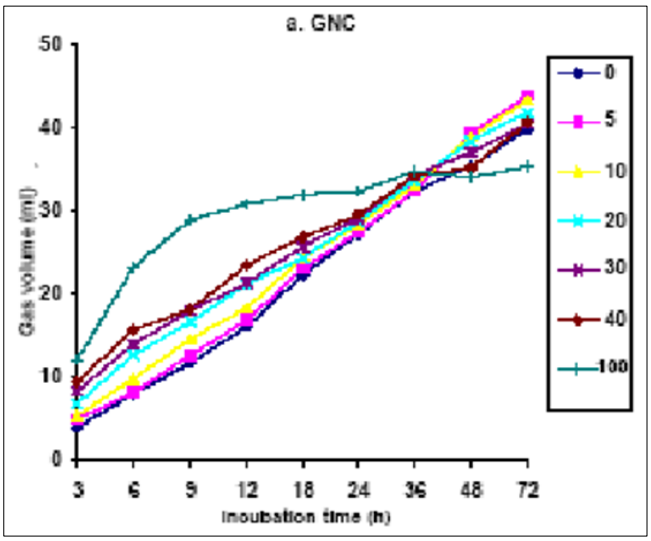

Fig 2: Gas production (ml) at different hours (h) of incubation of graded levels of groundut cake with wheat straw

Summary and Conclusion

In an experiment to investigate two approaches to predict optimal level of inclusion of groundnut cake for maximum true supplementary effect, with conventional short term animal feeding cum metabolic trials (Phase I) and *in vitro* gas production measurements to obtain kinetic and asymptotic values for gas production and complementing gas volume measurements with determination of truly fermented residue after a relatively short incubation of 24 hour, it was concluded that supplementation of ground nut cake at 5% level exerted significant effects though further levels improved the production parameters insignificantly. The *in vivo* experiments were correlated with *in vitro* gas production technique significantly. Hence, the cost effective *in vitro* trials could simulate the *in vivo* experiments.

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