puberty as glutathione level in hypothalamus is reported to reach a peak at puberty. Suppression of γ-GT transpeptidase activity by morphine in both the age groups suggests that this may be one of the mechanism(s) involved in suppression of gonadotropin secretion.


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# Inhibition of biogas production by isobutyric and isovaleric acids and their precursors

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**Isobutyric and isovaleric acids inhibit the production of biogas in the anaerobic fermentation of cowdung slurry at a concentration of 1000 ppm. Amino acids leucine and valine, whose metabolic products are isovaleric and isobutyric acids, respectively, when added to the fermenting media did inhibit the biogas production, but not to the extent free acids do, as some of the amino acids could have been utilized for protein synthesis.**

METHANE gas production under anaerobic fermentation of agricultural wastes and animal wastes is a complex multistep system involving different microorganisms, which first degrade cellulose and turn the product into methane with the help of methanobacterium present in the medium[1–3]. Many investigators have reported about the importance of volatile acids in the production of methane gas from cattle wastes. Varel *et al.*[4] observed that, under conditions of optimum methane production from cattle wastes, total volatile acid is the first to accumulate in large amounts by increasing the total volatile solids in the feed. The additive effect of addition of water hyacinth[5–7] and poultry waste[8] to cowdung enhanced the volatile fatty acid content. This was further confirmed using a two-phase fermenting system[9]. Hobson and Shaw[10] reported that volatile fatty acids acetate and butyrate were not inhibitory to methane production by *Methanobacterium formicium*, but propionate was under certain conditions. Kalle and Menon[11] reported that methanogenesis was inhibited by branched-chain fatty acids like isobutyric and isovaleric acids below ambient temperature. However, Kalle *et al.*[12] reported that normal mixed cellulolytic culture produces straight-chain fatty acids, which could be due to the presence of isovaleric-degrading bacteria present in marine sediment and sewage

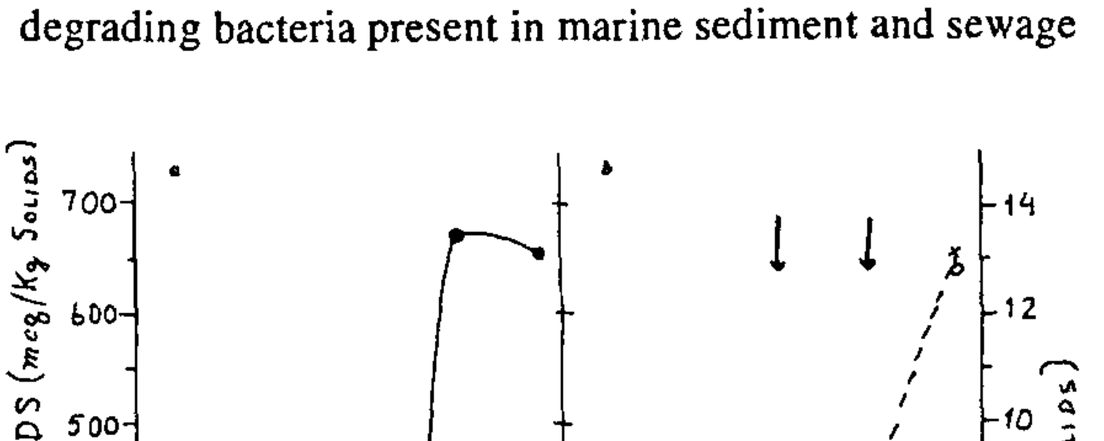


**Figure 1.** Effect of addition of isobutyric or isovaleric acid to the fermenting medium. Isobutyric acid (IBA) or isovaleric acid (IVA) was added on the 15th and 20th days of fermenting to a final concentration of 1000 ppm and the total amounts of volatile acids and biogas produced were measured. *a*, Control: ●----● total volatile acids; ●——● biogas produced. *b*, IBA or IVA added O----O IVA, total volatile acids; O——O IVA, biogas produced, ×----× IBA, total volatile acids, ×——× IBA, biogas produced

**Table 1.** Biogas production from cowdung in the presence of valine or leucine

| Treatment/ Days | 5 | 10 | 15 | 20 | 25 |
|---|---|---|---|---|---|
| | (l/kg solid) | | | | |
| Control | 0.22 | 1.36 | 3 49 | 12.33 | 12.11 |
| + valine (2000 ppm) | 0.15 | 1.05 | 2.89 | 11.06 | 10.94 |
| + leucine (2000 ppm) | 0 17 | 1 24 | 3.27 | 11.42 | 11.15 |
| SEM ± | 0.013 | 0 050 | 0 074 | 0 061 | 0.051 |
| CD (5%) | 0 046 | 0.176 | 0.259 | 0.212 | 0 178 |
| CD (1%) | 0 070 | 0 267 | 0.392 | 0.321 | 0 270 |

NB. Values are means of four replicates.

**Table 2.** Total volatile fatty acids in the medium in the presence of valine and leucine

| Treatment/ Days | 5 | 10 | 15 | 20 | 25 |
|---|---|---|---|---|---|
| | (meq/100 g solid) | | | | |
| Control | 7.79 | 15.53 | 25.86 | 14.20 | 13.19 |
| + valine (2000 ppm) | 7.86 | 16.40 | 29.20 | 18 40 | 17.33 |
| + leucine (2000 ppm) | 7.06 | 15 66 | 27 46 | 15.86 | 15.33 |
| SEM ± | 0.254 | 0.139 | 0.263 | 0 213 | 0.313 |
| CD (5%) | 0.880 | 0.482 | 0.912 | 0.739 | 1 084 |
| CD (1%) | 1.334 | 0.731 | 1.755 | 1.120 | 1.642 |

NB: Values are means of four replicates.

sludge[13]. Since the presence of inhibitors may also affect the rate of biogas production, the present study is directed towards the effect of branched-chain fatty acids and their precursors on biogas production.

Fresh cowdung was collected from the cattle farm of Rajasthan College of Agriculture, Udaipur. Volatile fatty acid in the fermenting media was estimated according to the method of Scarisbrick[14], by acidifying the sample with phosphoric acid to pH 2.5, diluting with equal volume of water and centrifuging to remove the solid material. Biogas production was measured by collecting the gas in an apparatus designed in this lab (Simlot, unpublished), which was connected to a digestor. The digestor contained 7.5% slurry of cowdung (total volume 400 ml) and was allowed to digest at $35 \pm 2^{\circ}C$. The gas was collected for 5 days and measurements made every 5th day.

The production of biogas depends on the medium, temperature and other anaerobic conditions of the medium and normally begins between days 3 and 12 at 55°C in cowdung fermentation[4]. As shown in Figure 1, in this case it started after 4 days and reached the maximum production of 13.46 l/kg solid on the 20th day in the control. Thereafter, the biogas production started declining to the level of 13.22 l/kg solid on the 25th day. The amount of total volatile fatty acids rose, reaching an optimum level of 262.9 meq/kg solid on the 15th day of fermentation and then dropping suddenly to 105.8 meq/kg solid on the 20th day. These observations about sudden spurt in biogas production from 15th to 20th day and concomitant drop in the total volatile fatty acid content confirms the earlier report that biogas production is dependent on the formation of volatile fatty acids[3]. On addition of branched-chain fatty acids like isobutyric and isovaleric acids (1000 ppm each) to the fermenting medium on the 15th day, when the biogas production had started picking up, it was found that gas production had gone down considerably, while at the same time the total volatile fatty acid content, instead of coming down, had started going up. Another dose of these acids on the 20th day had totally blocked the production of biogas. These results confirm the observations of Kalle and Menon[11] regarding the inhibitory effect of these acids on methane production.

Leucine and valine through a number of intermediate steps are metabolized to isovaleric and isobutyric acids, respectively. The presence of these amino acids in the fermenting medium can be used in two ways – either for the synthesis of new proteins or enzymes, or the excess may metabolize to isobutyric and isovaleric acids. The conversion of leucine and valine to isovaleric and isobutyric acids will inhibit the methanogenesis. Table 1 gives the volume of the biogas produced when leucine or valine was added to the fermenting medium. Initially, there was not much change in the amount of biogas produced in comparison to the control. But after the 15th day, biogas production was significantly reduced as compared to the control and remained at the lower level thereafter. The total volatile fatty acid content (Table 2), however, increased significantly over the control. Thus, these results indicate that the two amino acids are partially metabolized to their corresponding fatty acids, i.e. isovaleric and isobutyric acids, respectively, with the result that biogas production is inhibited.


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# Production of wheat haploids through embryo rescue from wheat × maize crosses

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**Appropriate hormone treatment using 2,4-D and gibberellic acid led to a high frequency (34.17%) of embryo formation in crosses of field-grown wheat strains with maize. Twenty-five days after culture on MS medium supplemented with casein (2000 mg/l) and amino acids, 30.95% of the embryos showed plant formation, 44.05% resulted in callus-like structures and the remaining failed to respond. The haploid nature of some of the regenerated plants was confirmed cytologically. Implications for wheat breeding are discussed.**

WHEAT is a premier food crop of worldwide importance. It is also a crop where conventional plant breeding has paid rich dividends, as epitomized by the green revolution. However, the current impasse in yield levels calls for application of newer techniques. A major hindrance to rapid genetic turnover in self-pollinated crops, such as wheat, is the inordinately long time (generally 8–10 years) it takes to develop stable, homozygous and ready-to-use material from a fresh cross. In this context, anther/microspore culture has often been proposed as a method for producing instant homozygous lines via haploidy. In wheat, wide hybridization followed by chromosome elimination serves as an alternative route to haploidy, e.g. wheat × *Hordeum bulbosum* and wheat × *Zea mays* crosses. The wheat × *Z. mays* cross is a relatively new system which offers distinct advantages such as freedom from genotypic specificity, the bane of anther culture approach as well as the wheat × *H. bulbosum* system (which is restricted to wheat genotypes carrying the kr crossability genes). Moreover, the system is less prone to gametoclonal variation owing to the absence of a dedifferentiated phase.

Zenkteler and Nitzsche[1] were the first to report microscopic, early-stage embryos in crosses between wheat and maize. However, the frequency of embryo formation was not specified, nor was evidence presented for their hybrid origin. Intrigued by this report, Laurie and Bennett[2] set out to study early post-pollination events in wheat and maize crosses under a CIMMYT project. They demonstrated the presence of both wheat and maize chromosomes in the zygotes and observed further that maize chromosomes were eliminated during initial cell divisions. Endosperm development ceases early or never occurs and embryos fail to develop to a size that can be readily rescued. Two years later, they reported[3] the recovery of the first haploid plants using the wheat × maize system by employing *in vitro* culture of wheat spikelets, 2 days after pollination. A method that bypassed spikelet culture was devised by Suenaga and Nakajima[4], who injected 2,4-D solution (100 ppm) into the uppermost internodes of wheat stems to sustain embryo growth on the plant itself, till the appropriate stage for embryo rescue.

The present study was conducted as a preliminary assessment of the applicability of the system to indigenous material grown under field conditions and to judge its potential as a plant breeding tool. The wheat material used represented advanced, agronomically relevant homozygous lines. (For actual plant breeding purposes, however, heterozygous material would be required.) Wheat ears were emasculated 3–5 days prior to anthesis by opening up the glumes and removing the anthers, unlike the conventional practice, which involves the cutting of the glumes to expose the androecium. Freshly collected pollen from a winter maize variety, Partap 1, was used for pollinations, 3–5 days after emasculation. The pollinated tillers were administered injections of 2,4-D solution (125 ppm) into the uppermost internode, daily in the evening, for three days after pollination. On the fourth day the ears were momentarily dipped in a solution of 2,4-D (100 ppm) + gibberellic acid (75 ppm). On an average, seed-like structures (Figure 1 *a*) were seen to develop in 86.71% of the wheat ovaries pollinated with maize (Table 1). Closer examination of the ears revealed that ovaries which failed to develop into seed-like structures were damaged by severe fungal infection. The presence of a large amount of maize pollen in the floret and moisture provided by hormonal (2,4-D) ear dip might have favoured this fungal growth. Thus, seed-like structures develop in all the viable florets. Obviously, all such seeds are not the result of fertilization and are probably formed due to hormone induced enlargement. Thus, actual seed setting could be assessed only after dissecting the seed-like structures and observing the presence/absence of embryos. Twenty developing seeds each of six genotypes 15 days after pollination with maize were specifically dissected for this purpose. No visual differentiation seemed to exist between seeds that carry an embryo