Effect of vinasse (condensed molasses solubles) on performance and meat chemical composition of Holstein male calves

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ABSTRACT

Twenty-four Holstein male calves (BW = 320 ± 48kg) were used to evaluate the effects of vinasse supplementation on growth, carcase and meat chemical composition and total-tract digestibility in a randomised complete block design. The calves were divided into four groups and allocated to four diets: a maize/barley-based diet with no added vinasse (C); a diet containing 5% (DM basis) vinasse (LV); a diet containing 10% (DM basis) vinasse (MV) and a diet containing 15% (DM basis) vinasse (HV). Amount of feed offered was recorded daily and the calves were weighed monthly and slaughtered after 4 months of trial. Dry matter intake was not affected significantly by treatments. Calves fed with C and LV diets had higher live slaughter weight, ADG, longissimus muscle area and lower feed efficiency than calves fed the MV and HV diets (p < .001). Digestibility of OM, EE and NDF were not different between C and LV diets (p > .05), but it was decreased as the level of vinasse increased to the level of 15% in HV diet (p < .05). No differences were detected in the NH3–N and molar proportion of rumen VFAs except for propionate, in which calves were fed the C and LV diets had higher concentration of propionate and total VFAs compare to those fed the MV and HV diets (p < .05). These results showed that vinasse can be included in the growing calves ration around 5% without adverse effects and would promote carcase composition.

Introduction

In the recent years, there has been growing interest in by-product management in food processing industry and utilising them as alternative feeds for animals due to enhanced environmental and economic concerns because most food by-products pose problems in the area of environmental protection (Tufarelli et al. 2013). Feeding agroindustrial by-/co-products and food residues to farm animals reduces the environmental impact of the food industry and improves profitability and valorisation of the agricultural by-products (Cooke et al. 2009) and is an efficient way to upgrade a low-quality material into high-quality food such as meat and reduces the dependence of livestock on feeds that could be consumed by humans (Bampidis & Robinson 2006). These products can also affect other major determinants of food quality for the modern consumer such as shelf life, sensory characteristics (appearance and eating quality), nutritional value, and health enhancers (Oltjen et al. 1964; NRC 1996).

Vinasse originating as the condensed molasses residue is a co-product of industrial production of alcohol, citric acid, yeasts, and other substances in the process of sugar production. It is also called condensed molasses solubles (CMS) (Fernandez et al. 2009). After the withdrawing the sugar fractions in the industry, the remaining organic non-sugar compounds and the mineral fraction in the molasses accumulate in the condensed remnant. This co-product is of no further interest in the manufacturing process, so the industry needs to find an efficient way to use it. Vinasse has the greatest polluting load of the effluents produced by alcohol distilleries because it presents oxygen bio-chemistry demand ranging from 20,000 to 30,000 mg/L vinasse (Leite 1999).

Vinasse can be used as a source of None Protein Nitrogen (NPN: including mainly betaine, glutamic acid and ammonia), in ruminants feeding. The main organic acids present in the vinasses are oxalate, lactate, acetate and malate, together with pyruvate. It is high in
potassium and sulphates content (Troccon & Demarquilly 1989; Stemme et al. 2005), so vinasse can be used for animal feed ingredient as a source of nutrients and minerals (Stemme et al. 2005; López-Campos et al. 2011).

Based on this information, the objective of this study was to evaluate effects of adding vinasse on performance, body composition, meat chemical composition and nutrient digestibility of Holstein growing calves.

Materials and methods

Animals and diets

The protocol for this study was approved by the University of Tehran Institutional Animal Care and Use Committee. Twenty-four Holstein male calves (average initial age 8.5 months, initial body weight 320 ± 48 kg with restriction of feed for 16 h) were randomly assigned to one of four dietary treatment groups with six calves per group as a randomised complete block design, and housed in individual pens as tie stall. The four experimental diets (Table 1) and their respective treatment groups were denominated as control (C, a maize/barley-based diet with no added vinasse), low level of vinasse (LV, 5% vinasse as DM basis), medium level of vinasse (MV, 10% vinasse as DM basis) and high level of vinasse (HV, 15% vinasse as DM basis). Diets were formulated to meet or exceed nutrient requirement using the Beef NRC model (NRC 1996) and to be isonitrogenous and isoenergetic. Table 2 contains chemical composition analysis of the four diets. The chemical composition and amino acids profile of vinasse used in this experiment are presented in Tables 3 and 4, respectively. Calves were gradually adapted to the experimental diets over 20 days. Diets were offered as total mixed ration (TMR), ad libitum and twice daily (0800 and 1500). Amount of feed offered was recorded daily, Before feeding each morning, feed refusals from each pen were weighed throughout the experimental period of 100 days in order to determine dry matter intake (DMI) and the feed conversion ratio (FCR). Clean drinking water was

| Table 1. Ingredients of diets fed to calves. |
|-------------------------------------------|
| Ingredient, % DM | C | LV | MV | HV |
| Alfalfa | 12.9 | 12.9 | 12.9 | 12.9 |
| Maize silage | 12.9 | 12.9 | 12.9 | 12.9 |
| Barley grain, ground | 28.6 | 28.6 | 28.6 | 28.6 |
| Wheat | 11.4 | 11.4 | 11.4 | 11.4 |
| Wheat bran | 28.3 | 22.1 | 16.1 | 10.1 |
| Soybean meal | 1.7 | 3.4 | 5.0 | 6.4 |
| Vinasse | 0.0 | 5.0 | 10.0 | 15.0 |
| Urea | 0.7 | 0.4 | 0.2 | 0.0 |
| Calcium carbonate | 1.0 | 1.0 | 0.4 | 0.1 |
| NaCl | 0.7 | 0.7 | 0.7 | 0.7 |
| Sodium bicarbonate | 0.7 | 0.7 | 0.7 | 0.7 |
| Vitamin mineral premix* | 1.0 | 1.0 | 1.0 | 1.0 |
| Zeolite | 0.7 | 0.7 | 0.7 | 0.7 |

*Premix contained: vitamin A: 600,000 U/kg; vitamin D: 200,000 U/kg; vitamin E: 200 mg/kg; antioxidant: 2500 mg/kg; Ca: 195 gr/kg; P: 80 g/kg; Mg: 21,000 mg/kg; Mn: 2200 mg/kg; Fe: 3000 mg/kg; Cu: 300 mg/kg; Zn: 300 mg/kg; Co: 100 mg/kg; I: 120 mg/kg; Se: 1.1 mg/kg.

| Item | % |
|------|---|
| Dry matter, % | 50.54 |
| Crude protein, % DM | 27.68 |
| Ash, % DM | 23.20 |
| Calcium, % DM | 2.98 |
| Phosphorous, % DM | 0.80 |
| Sodium, % DM | 3.01 |
| Potassium, % DM | 8.84 |
| Chloride, % DM | 3.20 |
| Glycerol, % DM | 3.35 |
| Betaine, % DM | 3.73 |

C: control diet with no added vinasse; LV: diet with low vinasse level (5%); MV: diet with medium vinasse level (10%); HV: diet with high vinasse level (15%); NE_{m}: Net energy for maintenance; NE_{p}: Net energy for growth; NDF: neutral detergent fibre.

| Amino acid | % |
|------------|---|
| Methionine | 0.12 |
| Cysteine | 0.12 |
| Lysine | 0.45 |
| Threonine | 0.45 |
| Arginine | 0.18 |
| Isoleucine | 0.06 |
| Leucine | 0.69 |
| Valine | 0.72 |
| Histidine | 0.18 |
| Phenylalanine | 0.3 |
| Glycine | 1.14 |
| Serine | 0.78 |
| Proline | 1.11 |
| Alanine | 1.89 |
| Aspartic acid | 1.32 |
| Glutamic acid | 13.05 |
| Total amino acids | 22.56 |
available for all calves and calves were weighed monthly.

**Slaughter and sampling**

Calves were slaughtered after 4 month of trial. During the 16 h previous to slaughter, calves had access to water but not to feed. Animal live weights were recorded before slaughter. After slaughter, gastrointestinal tract and internal organs were pulled out and carcasses were weighed to obtain the hot carcass weight and graded to determine 12th rib backfat thickness, and percentage of internal fat (the total weight of physically separable fat around the pelvic, heart, kidneys and gastrointestinal track as percent of carcass weight). The longissimus muscle (LM) area was measured between the 12th and 13th rib by tracings of LM using acetate paper according to the procedures of Naumann (1951). Following tracing, acetate paper tracings were measured using a compensating polar planimeter by three different person in three replication (a total of 9 planimeter measures per LM) and finally the average reported as square millimetre (mm²).

The 12th ribs were obtained from the right side of each carcase and used for carcase composition analysis. Soft tissues were dissected from the 12th rib section and were cut into small pieces, minced separately in a meat grinder and subsampled for chemical composition including ether extract, protein, ash and moisture content.

Total-tract apparent digestibility of nutrients was determined using acid-insoluble ash (Van Keulen & Young 1977) as an internal digestibility marker. On day 90–92, three faecal samples from rectum of each calf were collected over a 3-d period (one sample daily). Representative samples of each diet and all feed refusals were also collected on d 90–92 to determine diet chemical composition and nutrient intakes for the digestion period. Diets, feed refusals and faecal samples were stored at -20°C for later processing and analyses.

Rumen fluid sampling was performed on day 85 of trial, once from each calf at 3h after the morning feed allowance. Fifty milliliters of rumen content was collected through the rumen using a custom-designed stomach pump and a tube at one end into a 500 mL container. After collection, the ruminal contents were strained through 4 layers of cheesecloth and were analysed for pH immediately after filtration using a portable pH metre (Sentron, A102-003). Two 10-mL subsamples of ruminal fluid were then collected and mixed with chilled 25% (wt/vol) metaphosphoric acid (H₃PO₄) or 1% H₂SO₄ and stored at −20°C for later determination of VFA and NH–N, respectively (Chibisa et al. 2015).

**Chemical analyses**

Samples of feeds, refusals and faeces were oven-dried at 55°C for 72 h to constant weight and procedures described by the Association of Official Analytical Chemists (1990) were used to determine ash, crude protein and ether extract. Neutral detergent fibre (NDF) was completed according to methods of Van Soest et al. (1991), using the fibre analyser (Fibertec 1010). Samples of the right half-carcase were analysed for ash, crude protein (CP) and ether extract (EE) using AOAC (1990) procedures previously described.

Frozen ruminal fluid samples were thawed at room temperature and then centrifuged. Ruminal VFA were separated and quantified by gas chromatography (Varian 3700; Varian Specialties Ltd., Brockville, Canada) with a 15 m (0.53 mm i.d.) fused silica column (DBFFAP column; J and W Scientific, Folsom, CA). The ammonia content of ruminal samples was determined using the method described by Weatherburn (1967) modified to use a microtiter plate reader.

**Statistical analysis**

Data were analysed in randomised complete block design by the analysis of variance (ANOVA) using General Linear Model (GLM) procedure of SAS software V9.2 (SAS Inst., Inc., Cary, NC). Means were compared using Duncan’s new multiple range test (Duncan 1955).

**Results and discussion**

**Animal performance**

The influence of dietary treatments on intake and growth traits of calves are shown in Table 5. There were significant differences (p < .001) among treatments for live slaughter weight, average daily gain (ADG), and feed: gain ratio (FCR). Feeding MV and HV diets significantly decreased final body weight (BW) and ADG (p < .001) although dry matter intake did not differ significantly by the addition of vinasse. However, vinasse increased DMI numerically and led to significantly increase of FCR (p < .001) while feeding MV and HV diets. In agreement with our results López-Campos et al. (2011) reported that the addition of 100 or 200 g of vinasse per kg of concentrate for fattening lambs reduced feed intake and growth rate and increased the feed: gain ratio.
Carcass measurements and meat chemical composition

There were significant differences in carcass measurements including hot carcase and internal fat weight and longissimus muscle area (Table 6). Calves that were fed the HV diet had lighter carcases compared to those fed the C diet. Internal fat weights in kg basis significantly decreased, as the level of vinasse in the ration increased and reached to the level of 15% in HV diet. In accordance with the effects of treatments on the hot carcase weight, calves that fed the MV or HV diets had smaller LM area compared to those fed the C or LV diets. Carcass yield and internal fat as a percentage of body weight did not differ among treatments. There were significant differences in meat chemical composition of longissimus muscle (Table 6); the percent of EE and ash were significantly increased (p < .05) and CP percent was significantly decreased due to the feeding of MV and HV (p < .05). Treatments had no significant effect on moisture content of muscle (p > .05).

In agreement with our results, Potter et al. (1985a) and Yalcin et al. (2010) observed leaner carcases from steers supplemented with 100 kg condensed molasses solubles (CMS) and significant reduction of the kidney, heart and pelvic fats. The lipolytic effect of the vinasse would probably be related to its high betaine content (Table 3). Indeed, the involvement of betaine in lipolysis offers interesting challenge for lean meat production in meat-producing cattles (Fernandez et al. 2000) and some researchers indicated a positive effect of betaine on protein metabolism (Mitchel et al. 1979; Puchala et al. 1995). It has been demonstrated that betaine supplementation significantly reduced the subcutaneous fat at the 12th and 13th ribs in female lambs. Yalcin et al. (2010) and Fernandez et al. (2000) observed an 11% reduction of subcutaneous fat thickness in betaine-supplemented lambs.

Apparent digestibility

Vinasse had significant effects on nutrient digestibility of diets (Table 7); in overall view, digestibility coefficients (except for CP) were not different between C and LV diets but feeding calves with greater level of

| Table 5. Effect of dietary treatments on performance of calves. |
|---------------------------------------------------------------|
| Treatments | Item | C | LV | MV | HV | SEM | p Value |
|------------|------|---|----|----|----|-----|--------|
| Live slaughter weight, kg | 417.5 | 415.8 | 403.2 | 401.7 | 9.56 | .001 |
| ADG, kg | 1.26 | 1.24 | 1.02 | 0.99 | 0.12 | .001 |
| DMI, kg/d | 7.57 | 7.71 | 7.87 | 8.08 | 0.23 | .422 |
| FCR | 6.02 | 6.26 | 7.72 | 8.11 | 0.22 | .001 |

C: control diet with no added vinasse; LV: diet with low vinasse level (5%); MV: diet with medium vinasse level (10%); HV: diet with high vinasse level (15%). Different superscripts following means in the same row indicate differences at p < .001.

| Table 6. Effects of the vinasse on carcase characteristic and longissimus muscle composition of calves. |
|---------------------------------------------------------------|
| Treatments | Item | C | LV | MV | HV | SEM | p Value |
|------------|------|---|----|----|----|-----|--------|
| Hot carcase weight, kg | 220.5 | 217.5 | 213.4 | 211.5 | 6.16 | .017 |
| Carcass yield, % | 52.81 | 52.36 | 52.90 | 52.63 | 0.48 | .872 |
| Internal fat, % | 2.85 | 2.63 | 2.55 | 2.52 | 0.12 | .377 |
| Internal fat, kg | 6.28 | 5.73 | 5.47 | 5.38 | 0.14 | .018 |
| LM area, cm² | 67.1 | 66.4 | 62.9 | 62.7 | 0.72 | .001 |

| LM chemical composition | Moisture, % | 623 | 625 | 637 | 634 | 10.70 | .231 |
| Ether extract, % | 52 | 537 | 541 | 543 | 5.89 | .039 |
| Crude protein, % | 248 | 239 | 234 | 227 | 4.51 | .035 |
| Ash, % | 209 | 214 | 221 | 223 | 4.58 | .046 |

C: control diet with no added vinasse; LV: diet with low vinasse level (5%); MV: diet with medium vinasse level (10%); HV: diet with high vinasse level (15%). Different superscripts following means in the same row indicate differences at p < .001.

| Table 7. Effects of the vinasse on nutrient apparent digestibility. |
|---------------------------------------------------------------|
| Treatment | Item | C | LV | MV | HV | SEM | p Value |
|------------|------|---|----|----|----|-----|--------|
| Dry matter, % | 73.2 | 75.1 | 70.2 | 69.3 | 1.34 | .041 |
| Organic matter, % | 75.4 | 77.9 | 72.5 | 72.2 | 1.19 | .038 |
| Crude protein, % | 77.4 | 76.1 | 75.3 | 75.0 | 1.32 | .422 |
| Ether extract, % | 72.3 | 74.8 | 73.8 | 67.9 | 1.40 | .035 |
| NDF, % | 48.0 | 51.6 | 44.1 | 42.3 | 1.39 | .019 |

C: control diet with no added vinasse; LV: diet with low vinasse level (5%); MV: diet with medium vinasse level (10%); HV: diet with high vinasse level (15%). Different superscripts following means in the same row indicate differences at p < .05.

aNeutral detergent fibre.
vinasse (MV and HV diets) affected digestibility of nutrients dramatically.

By increasing vinasse from 0 (C diet) to 15% (HV diet), the digestibility of dry matter (DM), organic matter (OM), ether extract (EE) and neutral detergent fibre (NDF) decreased significantly \((p < .05)\). Treatments had no effect on CP digestibility \((p > .05)\). In contrast to our results, Fernandez et al. (2009) reported that the apparent digestibility of NDF tended to be higher when vinasse was added to sugar beet pulp at the level of 13% in diet of lambs. Also, they reported no differences among treatments for DM, OM, CP and ADF digestibility coefficients. Also, Kania et al. (1983) reported higher crude fibre digestibility in CMS-fed animals.

These effects can be related to the fact which CMS is a condensate of a fermentation effluent and contains many factors that potentially could be stimulatory to rumen microorganisms. Also, Hannon and Trenkle (1990) by in vitro studies showed that the nitrogen in CMS was available to mixed cultures of rumen bacteria for the synthesis of amino acids and dry matter digestion. Feeding bulls by higher CMS (more than 5%) resulted to depression of DM, OM and NDF digestibility. Hannon and Trenkle (1990) mentioned that deleterious effects of higher concentration of CMS (10% and 15%) can be caused by the high sulphur content.

**Characterization of ruminal fermentation**

There were significant differences among diets in the concentrations of propionate, acetate: propionate ratio, total VFA and pH (Table 8). Propionate and total VFA concentration decreased significantly \((p < .05)\), as the proportion of vinasse increased in diets, but the acetate: propionate ratio and pH increased significantly \((p < .05)\). There was no significant difference among diets in ruminal \(\text{NH}_3\)–N concentrations \((p > .05)\).

In agreement with our results, Potter et al. (1985b) showed that Steers fed 0% and 5% CMS had higher propionic acid concentration than those fed 10% and 15% CMS. Also, they reported greater acetate: propionate ratio for the two higher levels of CMS. López-Campos et al. (2011) reported that feeding vinasse in fattening lambs significantly increased ruminal pH. In contrast to our results, Fernandez et al. (2009) reported no significant differences between control diet and CMS containing diet for volatile fatty acid (VFA) concentration, ruminal pH and ammonia nitrogen in merino ewes fitted with ruminal cannulae. The effect of vinasse on ruminal pH may be related to high potassium content of vinasse, which is an alkali mineral and can increase pH values.

Chalupa (1980) related the greater feed:gain ratio when vinasse was fed to cattle which resulted in decline in production of propionate in relation to acetate. In the current study, however, inclusion of vinasse to diets caused decrease in feed price but feed conversion increased simultaneously and resulted to greater feed cost per kg of gain for MV and HV diets compare to C and LV diets. In agreement with our results, Potter et al. (1985b) showed that dry feed/unit gain for 0% and 5% CMS was not different, but 10% and 15% CMS increased dry feed required per unit gain.

There were no significant differences among diets in the concentration of butyrate and valerate. Also, Potter et al. (1985b) showed that valeric and iso-valeric acid concentrations were not influenced by treatments. In contrast to our results Wahlberg & Cash (1979) and Hannon and Trenkle (1990) reported that the addition of vinasse increased the ruminal \(\text{NH}_3\)–N concentration.

**Conclusions**

Based on the results of current experiment, the inclusion of 5% vinasse in the diet of growing calves have positive effects on supply of N, modify body composition and produce leaner meat. Also, our results showed no negative effects of inclusion 5%
vinasse on feed intake, ADG, FCR, carcase characteristic and nutrient digestibility. In comparison to 5%, inclusion of 10% and 15% of vinasse had negative effects on performance including ADG and FCR and reduced nutrient digestibility and total VFA production.

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