pH EFFECT ON THE PRODUCTION OF CONJUGATED LINOLEIC ACID (CLA) AT BIOREACTOR LEVEL

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Introduction. Currently, the interest of consumers regarding nutritional quality is increasing (1). Substances such as conjugated linoleic acid (CLA) are widely studied due their beneficial effects on diseases related to lifestyle and cancer (2, 3, 4). CLA is produced by microbial isomerization of linoleic acid in the rumen, or Δ-9-trans-vaccenic acid (t-VA) desaturation process, which happens in most mammals' bodies, including humans (5). This production occurs at very low concentrations; hence, seeking better yield alternatives is important.
This research was focused on the study of the pH effect on the CLA production at bioreactor level.

Methods. Six extruded cattle feed (alfalfa or bean based, enriched with canola or sunflower oil), were developed (6). The fermentations were performed at the first stage of *in vitro* digestibility analysis technique of Llamas and Tejada (1990). Subsequently, lipid extraction was performed based on the Folch method (1957), modified by Bligh and Dyer (1959). Methylation of fatty acid esters was performed following AOAC method (1969). Quantification of fatty acids was performed by gas chromatography following Roach *et al.* method (2002). This research was conducted in two stages. In the first stage, fermentations were allowed to run without pH adjustment, in the second stage, four different pH values remained constant.

Results. In the first stage, pH was not controlled during fermentation time, three out of six diets, showed greater CLA concentration at extracellular pH values between 6.0 and 6.5 for 6 h of fermentation (Table 1).

Table 1. CLA percentage and pH batch fermentation of diets with greater CLA production

Diet	CLA (%)	pH
Bean-Canola	5.77±0.193^{a}	6.38±0.014^{bc}
Alfalfa-Canola	3.11±0.031^{b}	6.47±0.035^{b}
Bean-Sunflower	2.79±0.113^{bc}	6.37±0.021^{c}

± standard deviation. ^{ac} Different letters in the same column indicate statistically significant differences ($p \leq 0.05$)

In the second stage, it was sought to maintain constant pH, tests were performed with alfalfa extruded diet with 5% of canola oil. For this stage, different buffer proportions were tested to maintain constant pH.

Conclusions. In the first stage, highest CLA production showed in fermentation times of 6 h and very limited pH values: 6.18, 6.57 and 6.37, therefore, it is required to determine the optimum pH value for CLA production at bioreactor level. In the second stage, contrary to what might be expected, increasing buffer solution volume, treatments' pH with higher buffer proportions descended further than the treatments with minor buffer proportion, indicating greater microorganisms activity, which creates a pattern to assume that maximum CLA concentration can be reached in less fermentation time.

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