Plant Archives Vol. 21, Supplement 1, 2021 pp. 1256-1261 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
doi link : https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.198

IN VITRO DIGESTION PRODUCTS OF RATION CONTAIN DIFFERENT LEVELS OF FABA BEANS TREATED WITH FORMALDEHYDE USING ARABI SHEEP RUMEN LIQUID

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ABSTRACT

Faba beans are a major protein source for both ruminant and non-ruminant species. A current study focuses on the *in vitro* digestion of five dietary regimens with different levels of faba bean (*Vicia faba*), 0 (control), 5% (T2), 5% + formalin (T3), 10% (T4), and 10% + formalin (T5). The *in vitro* fermentation characteristics were studied for 48 h using rumen liquid from sheep as inoculum. Gas production during the period of incubation was recorded. The digestibility of dry matter and the volatile fatty acid were calculated at the fermentation end. Evident in vitro degradability of DM (ivTDDM), OM degradability (ivOMD) was decreased by the degree of expansion as faba replaced soya bean meal at 10% especially with formalin. Microbial weight (g / kg DM) factor of partitioning (PF), and ammonia concentration were slightly lower by adding 10 percent of faba beans with formalin or without. Metabolizable vitality (ME) (MJ/ kg DM), volatile fatty acid and microbial production efficiency (MPE) were more common in all types of diets. In conclusion replacing soybeans with faba beans did not lead to significant changes to the rumen fermentation and digestion of sheep nutrients. The findings of this study support the likelihood that faba bean might be used in ruminant nutrition with the benefit that it has a distinct nutritional characteristic that affect the parameters of *in vitro* fermentation.

***Keywords*:** Faba beans, in vitro gas production, microbial mass.

Introduction

A leguminous plant (a member of the pea family), is one of the richest sources for protein, energy, minerals and vitamins that could use in human and animal nutrition (Ahmed and Hasan, 2014). Grain legumes, especially faba beans are rich in valuable protein sources which mainly used in many animals' nutrition ratio; however, it should also be taken into consideration the high content of starch (Musco *et al.*, 2017). In animals concentrating feed ratio, faba beans seeds could use as a good alternative source to soybeans seeds (Nolte *et al.*, 2020).

Historically, legumes have been known as the earliest humans domesticated plants (Ahmed and Hasan, 2014). China, Australia, France, Egypt and many other countries around the world have realized the importance of growing faba beans for humans and animals' consumptions (Pelagalli *et al.*, 2020). Also, leguminous plants have a positive effect on soil fertilization. They play a fundamental role in crops rotation. Generally, faba beans as a one of Peas family enhance soil fertility and decrease the required amount of nitrogen fertilization by fixing the atmospheric nitrogen throw-out the soil rhizobia bacteria (Jensen *et al.*, 2010).

The faba bean has been a promising alternative to soybeans as a protein source used in animal nutrition as the high available protein source content. However, the endogenous pyrimidine glycosides vicin and convicin (VC) in faba beans are considered anti-nutritional factors that, limit the use of faba beans in human and animal nutrition (Nolte *et al.*, 2020). Tannin is another main anti-nutritional factor besides VC. The existence of tannins in the faba bean has shown a reduction of protein digestibility beside apparent metabolizable energy. There is no definite effect of faba beans on animal health and efficiency, and the findings of numerous studies differ greatly.. In dairy cows, Brunschwig and Lamy (2002) reported that using 30% of faba beans in the concentrated feed did not change milk yield and components, and feed consumption. Experiments on sheep by Liponi *et al.* (2009) fed high levels of raw faba beans did not show any negative effects on diet palatability and digestibility.

The utilization of an in vitro gas strategy in assessing feedstuffs (through the estimation of factors like methane, microbial mass and short-chain unsaturated fatty acids (SCFA) is a successful and strong method of evaluating the loss of calories, microbial and feed nitrogen supplied to ruminants (Anele *et al.*, 2011).

The present study aimed at the effect of using different levels of either treated or not treated faba beans with formaldehyde on in vitro digestion and some rumen parameters of sheep.

Materials and Methods

Four rations were used in this study (table, 1) using different levels of faba beans (0, 5, or10%) either with formalin or without. Faba bean treated with formaldehyde 4% as 1 liter/10 kg of faba beans. The rumen fluid was taken

from the sheep slaughtered at the Basra province slaughterhouse. Samples were transferred directly to the laboratory and filtered through four layers of the cheese fabric. A total of 300 ml of rumen liquid was taken for each treatment, 2 g of the diet is taken, 5 ml of rumen liquid is added with 1 ml of artificial saliva solution and place in a 20 ml syringe and control well after monitoring the gas output after 1 hour, 3 hours, 6 hours, 12 hours, 18 hours, 24 hours, 48 hours were measured. After incubation in the rumen liquid, the feed samples were washed three times for 5 min and dried in forced ventilation at 45°C.

Table 1 : Rations used in the study

Treatments	Barley	Wheat bran	Soya beans	Fab beans	Vitamin and minerals
	%				
Control	52	35	10		3
2nd treatment	52	35	5	5	3
3rd treatment	52	35	5	5 (with formalin)	3
4th treatment	52	35		10	3
5th treatment	52	35		10 (with formalin)	3

Table 2 : Chemical composition (%) of studied rations on a dry matter basis

Feed	Dry matter	Protein	Ether Extract	Crude fiber	Ash	Organic matter	NFE
Barley	92.85	10.72	1.42	6.50	3.82	89.03	70.39
Wheat Bran	90.42	15.86	4.05	10.63	4.99	85.43	54.89
Soya bean meal	91.70	45.90	7.21	2.51	6.14	85.56	39.66
Faba beans	86.60	29.00	1.40	6.70	3.90	82.70	53.70

NFE, Nitrogen Free Extract.

To correct the rumen fluid gas production, blank syringes (no samples) were used. Data is fitted to the exponential model of Orskov and McDonald (1979), $y= a+ b (1-exp^{-ct})$, Where: y is the gas production volume at time 't' (h), a is the produced gas from the soluble portion immediately (ml), b is the gas production from the insoluble portion (ml), and c is the rate of gas production constant for the insoluble fraction b (h)after subtraction of gas input from blanks. Syringes without (blanks) fermentation samples were used to let the gas directly formed from the rumen fluid to be fixed.

Half-time (t1/2) asymptotic gas output (b; mL) was determined as: t1/2= (ln2 / c). Metabolizable energy, using the Menke *et al*. (1979) method: ME (MJ)= 2.20 + 0.136 GP + 0.057 CP+ 0.0029 CF2. And the digestibility of organic matter: ivOMD %= 14.88 + 0.889 GP + 0.45 CP + 0.0651 XA. Where: GP: amount of total gas after 24 hours, CP: crude protein, CF: crude fat and XA: ash. Using the equation defined by Getachew *et al*. (2004), the short chain fatty acid (SCFA) output (mmol) was calculated: SCFA= 0.0239 GP-0.0601.

The fermentation parameters of the rumen fluid (pH and ammonia N) were measured at the end of incubation at t1/2, 24 h and 96 h (Hu *et al*., 2005).

Truly *in vitro* degraded dry matter (*iv*TDDM)

Truly dry matter degraded in vitro (ivTDDM) has been calculated using techniques defined by Anele *et al*. (2011). Substrate-specific t1/2 was calculated after the initial 96 h gas run and a layer specific t1/2 was calculated.

At t1/2 and 24 h, the incubations were cut off and gas volume was registered. Anele *et al* (2011) and Van Soest *et al.* (1991) approaches were used to measure the true degradability of diet substrates at t1/2 and 24 h (ivTDDM = incubated feed (DM) − residue (DM) retrieved in incubated crucibles / feed (DM)). High-speed centrifugation (20,000 g) of incubation residues at 20◦ C for 30 min (Blummel and Lebzien, 2001) was determined in vitro apparent degraded DM at t1/2 and 24 h after placement in iced cubes (about -4◦ C) to avoid fermentation. Blanks have also been centrifuged and residues have been measured and used to fix ruminal inoculum residues. The obvious deteriorated DM coefficient in vitro was determined as: incubated feed (DM)- [pellet (DM)-blank pellet (DM)]/incubated feed (DM). In vitro apparent degraded DM was determined by high-speed centrifugation (20,000 g) of incubation residues at 20° C for 30 min (Blummel and Lebzien, 2001) after placement in iced cubes (about -4 ºC) to avoid fermentation. Blanks have also been centrifuged and residues have been weighted and used to fix the ruminal inoculum residues. The in vitro apparent DM degradability coefficient was computed as: Feed (DM) incubated - [pellet (DM)−blank pellet (DM)]/Feed (DM) incubated.

Partitioning factor (PF) was measured as: PF= *iv*TDDM (mg) / GP_{24} (mL). The production of in vitro microbial mass was measured using the formula of Blummel *et al*. (1997) as: microbial mass (mg)= ivTDDM (mg)-(GP24 (mL)* 2.2), where: 2.2: stoichiometric factor due to the quantities (mg) of O, H, and C needed for 1 mmol production from SCFA and related 1 mL gas.

Statistical analysis

Data were analyzed by one-way anova within the statistical program SPSS (2016). Means differences were recognized as significant (P<0.05) by using Bonferroni test within the same statistical program.

Results and Discussions

Figure (1) indicates the fermentation kinetics during incubation of the various diets. Inclusion of concentrate did not impact gas output (mL/g OMD), regardless of the presence of FABA ($p > 0.05$). However, based on the existence of FABA the parameters of the fermentation kinetics were influenced differently. Potential output of gas only decreased with the addition of FABA concentrate and formalin ($p<0.05$). The rate of gas output c appeared to increase with the inclusion of the control concentration ($p < 0.10$), while with the inclusion of FABA concentration it decreased (Table, 3). Gas occurs directly from microbial oxidation of feeds and partially from acid buffering arising from fermentation.

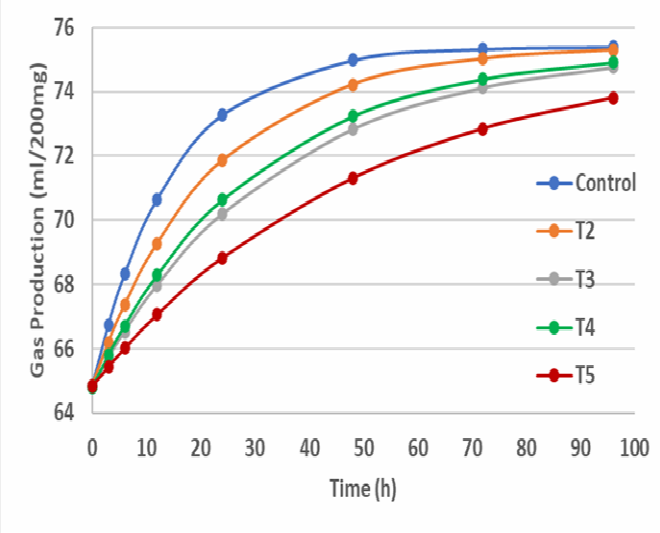

Fig. 1 : The profile of *in vitro* gas production of the control and faba treatments

Table 3 : In vitro fermentation characteristics of different levels of faba bean

Degradation Parameters	Treatments				
	control	T2	T3	T4	T5
A	64.831	64.849	64.807	64.793	64.839
B	10.575	10.590	10.558	10.530	10.549
C	0.0667	0.0451	0.0297	0.0336	0.0199

T2=5% faba beans+5% soya beans meal, T3=5% faba beans+5% soya beans meal with formalin, T4= 10% faba beans, T5=10% faba beans with formalin, a= the gas production from the immediately soluble fraction (ml), b= the gas production from the insoluble fraction (ml), and c= the gas production rate constant for the insoluble fraction b (h).

Vicia faba spp and other legume seeds can be considered a very good source of protein for ruminants. The *in vitro* fermentation characteristics for the faba beans tested in this study fall within the literature range recorded (Calabro *et al*., 2009). Favored patterns of *in vitro* fermentation in terms of degradability, kinetics, gas and VFA production, high energy content and low structural carbohydrate level in the tested faba beans grains. In a preceding study (Calabro *et al*., 2009), In vitro incubation was carried out for different varieties (six) of faba beans with buffalo rumen fluid, with higher degradability values, but slower fermentation kinetics were reported. Such data suggest that the in vitro fermentation process can be influenced by many factors (plant types, donor animal species, incubation time). As described in a preceding study (Musco *et al*., 2017), the carbohydrate fractions affect the kinetics of fermentation differently: starch promotes a more intense and rapid process, while structural carbohydrates trigger a slower and less stable fermentation. Lei *et al.* (2018) reported that the dry matter degradability of feed ingredients in rumen increased with increasing incubation time, the degradation of energy feed ingredients was on an uptrend prior to 24 h incubation and then the process slowed down, while protein feed ingredients were at 36 h, which was on an uptrend all the time. This suggested that feed ingredients for energy feed ingredients were almost entirely digested at 24 h and 36 h for protein feed ingredients, respectively. The particularly slow in vitro fermentation kinetics (lowest value potential gas output) was likely due to the high ADL content that limited the access of microorganisms to cell material, decreased nutrient degradability and slowed fermentation rate; as demonstrated, also by the undesirable correlation between lignin material and degradation of in vitro organic matter (He *et al*., 2020).

Data from the processing of laboratory gas revealed that the coefficient of digestion of organic matter and the rate of digestion of dry matter varied significantly ($p<0.05$), while the concentration of total volatile fatty acids and the amount of energy produced between treatments did not vary (Table 4). Using formalin with 10% faba lowered ($P<0.05$) all parameters.

Table 4 : The concentration of gas production, organic digestion rate, metabolizable energy and total fatty acid of studied rations

Rations	GP	ivOMD	ME	*iv*TDDM	SCFA
Control	24.66	611.60a	11.76	700.00a	37.42
2nd treatment	25.67	573.98b	11.66	670.00b	37.00
3rd treatment	25.00	538.80c	11.49	640.00c	36.79
4th treatment	25.25	546.89c	11.35	650.00c	36.54
5th treatment	24.90	511.73d	11.32	620.00d	36.37

GP=gas volume (mg/200mg), ivOMD=organic matter digestion (mg), ME (MJ/kg DM) =metabolizable energy, ivTDDM=real organic matter digestion (mg), SCFA= short chain volatile fatty acid (meq/L). Means with different letters vertically differ significantly at (P<0.05).

The increase in the production of gas may be attributed to the growing abundance of nutrients in the concentrated diet (Ahmed and Abdel, 2007) or to a decline in the quality of lignin on the cell wall attributed to concentrate being 100% of the diet (Frei, 2013; Al-Masri, 2009). The results were with an agreement with the results of Kumari *et al*. (2012), Seshaiah *et al*. (2014) and Reddy *et al*. (2015), who found an increase in gas production of roughages ration when replaced by concentrate. A similar result of gas production with digestion rate of organic matter, energy content due to the decreased level of cellulose, hemicellulose, and lignin (digestion reducer) in the diets (Frei, 2013; Al-Masri, 2009). Availability of nutrients for rumen microorganisms also improve organic matter digestibility (Ahmed and Abdel 2007). These results in accordance with those of Reddy *et al.* (2015), Polyorach *et al.* (2014) and Khanum *et al*, (2007), who found an improvement in digestibility of organic matters of concentrated diets. The concentration of total volatile fatty acids increased, which reflected an increase in performance of microorganisms. This result was similar to those of Getachew *et al.* (2004), who concluded that total volatile fatty acids correlated significantly and positively with the increase in gas production.

Table (5) showed significant differences (P<0.05) in ammonia nitrogen, the partitioning factor and microbial mass, where the group that did not add formalin was exceeded, regardless of faba percentages in comparison with control group. Whereas, the microbial production efficiency among the different treatments were mostly similar even. The results show that ammonia production decreased linearly with faba added to the rations. While the quantities produced of the 5% of beans or soybeans groups are equal to control.

Table 5 : Ammonia concentration (AC), partitioning factor (PF), microbial mass (MS) and microbial production efficiency (MPE)

Rations	AC (mg/100ml)	PF mg/ml	MS (mg)	MPE %
Control	58.00a	2.28a	44.12a	76.97
2nd treatment	56.87ab	1.99ab	43.26ab	76.41
3rd treatment	55.21b	1.68ab	42.00ab	75.87
4th treatment	54.36b	1.74ab	41.35b	76.09
5th treatment	55.18b	1.44b	41.97b	75.58

Means with different letters vertically differ significantly at (P<0.05). MPE= 67.9- 2.49ivTDDM (Oba and Allen, 2003)

Ammonia production decreased significantly (P<0.05) with the addition of formalin, due to the inhibition of protein degradation by rumen microorganisms as it was recorded that formalin reduces protein degraded by 30-40% in the rumen (Saeed, 2011). This result was in agreement with those of Kumari *et al.* (2012) and Reddy *et al.* (2015), they found an increase in ammonia concentration of concentrated diet in comparison with roughages due to the degradation of high proportion of high protein level of concentrations especially when level of energy is adequate to microorganisms needs (Thirumalesh and Krishnamoorthy, 2013). The variability of the number of rumen microorganisms of different rations mainly depends on rumen environment as the pH and the availability of important nutrients for growth (carbohydrates and nitrogen) which translated into a sharp growth of microbes (Thirumalesh and Krishnamoorthy, 2009). Increase in microorganisms number leads to improvements in digestibility of organic matter, as there is a significant positive correlation between the level of nutrients in the rumen and number of microorganisms (Thirumalesh and Krishnamoorthy, 2009).

Pelagalli *et al.* (2020) showed that nitrogen is degraded by more than 85 percent. The average theoretical nitrogen degradability for three separate faba bean cultivars was 92% in the rumen in 2 h. For this reason, in an earlier study (Cutrignelli *et al*., 2008), it was proposed that a protein source rich in rumen undegradable protein should be paired with faba beans immediately after weaning. Azarfar *et al.* (2008), 72 h incubating refined grains of a variety of Vicia faba minor using the technique of in vitro gas processing with dairy rumen liquor cows, find a lower gas output value and a lower volatile fatty acid supply. Dietary protein lower ruminal degradation in the small intestine may have poorer digestibility than microbial protein, which is more costly than ruminal degradation. The microbial protein in the small intestine is easily digestible, and its amino acid composition is similar to what ruminants want (Arbabi *et al*., 2017).

Conclusion

The findings of this study support the likelihood that faba bean can be used in ruminant nutrition with the benefit that it has a distinct nutritional characteristic that affect the kinetics of in vitro fermentation and may also impact their use in vivo. Replacing soybeans with faba beans did not lead to significant changes to the rumen fermentation and digestion of sheep nutrients.

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