EFFECT OF USING PROBIOTICS ON ARABIAN CAMELS (*Camelus dromedarius*) FEEDING: AN *IN VITRO* STUDY.

Aml, M. Arafa[1,2]; H.M. Gado[1]; M.M. Ghandour[2]; H.M. Metwally[1] and A.R. Askar[2]

[1]*Animal Production Department, Faculty of Agriculture, Ain Shams Univ., Shobra El-Khima, 11241, Cairo, Egypt.*

[2]*Animal and Poultry Nutrition Department, Desert Research Center, El-Matareya 11753, Cairo, Egypt.*

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SUMMARY

Three types of probiotics (*Ruminococcus flavefaciens*, *Bacillus subtlis* and *Lactobacillus acidophilus*) were used (single or combined) to study their effects on gas production (GP), *in vitro* dry matter disappearance (IVDMD%), degradability parameters (a, b and c) and predicted dry matter intake using rumen liquor of Arabian camels. All probiotics were used in concentration of 2 ml/Kg DM. The tested feed consisted of alfalfa hay (*Medicago sativa*) and concentrate feed mixture (CFM) in total mixed ration (TMR) with roughage concentrate ratio (R:C) equal to 60:40. Group1 (control without probiotic), group2 (*Ruminococcus flavefaciens* (ZAD)), group3 (*Bacillus subtlis*) and group4 (ZAD+ *Bacillus subtlis* + *Lactobacillus acidophilus*). There was no significant difference in gas production at 24h and overall mean of gas production through experimental groups. Group4 (probiotics combination) was the highest among the tested groups in IVDMD%, degradability parameters and predicted dry matter intake ($P< 0.05$). That may be due to positive synergistic effects of probiotics combination.

Keywords: *Camel, gas production, in vitro, probiotic, Zad, Bacillus subtlis and Lactobacillus acidophilus.*

INTRODUCTION

Arabian camels (*Camelus dromedarius*) (synonym for one humped camel or dromedary) play essential role in pastoral (Bedouin) communities by providing food (milk and meat), draft animal power and transportation tool due to their highly adaptation to the desert conditions.

Rahimi *et al*. (2022) showed that one of the best scenarios to adapt to the negative effects of climate changes (such as severe and long drought periods) in north sub-Saharan Africa is shifting from cattle to camel and goat farming. That will increase milk production, decrease the demand of water and feed for animals and decrease methane production from animals.

Uncontrolled uses of antibiotics as growth promoters in animal production sector over decades led to rise antimicrobial resistance that considered as a big challenge for human and animal health. So, using alternative resources (probiotic, prebiotic, phytogenic, organic acids …etc.) is highly recommended worldwide (Seal *et al*., 2013 and Aslam *et al*., 2021).

ZAD® is a liquid anerobic probiotic (*Ruminococcus flavefaciens*) with multi exogenous enzymes (cellulase, xylanase, amylase and protease) which are designed to enhance rumen function and whole digestive tract digestion (Gado, 2020). Moreover, Sayed *et al*., (2023) reported that using ZAD as probiotic with TMR (40:60 R:C) significantly improved DMD, NDFD and ADFD % compared to control group (*in vitro* study). Additionally, Ashour *et al*., (2023) reported that using ZADO (powder form of ZAD) in growing Arabian camels as feed additives resulted in higher growth rate in camels followed 628, 804 and 910g/day for zero, 20 and 40g ZADO/head/day, respectively.

Even though camel is functionally ruminant, it has a unique characteristic in rumen and whole digestive tract digestion. So, this study was aimed to investigate the role of different probiotics (single or combined) in *in vitro* system such as gas production and *in vitro* dry matter disappearance (IVDMD%) using rumen liquor of Arabian camels.

MATERIALS AND METHODS

Experimental ration and feed additives (Probiotics):

Alfalfa (*Medicago sativa*) hay and concentrate feed mixture (CFM) were used to formulate total mixed ration (TMR) with roughage concentrate ratio (R:C) equal to 60:40 (to stimulate milk production feeding). The chemical composition of alfalfa hay, CFM and calculated chemical composition of TMR are shown in Table (1). Three types of commercial liquid probiotic feed additives (ZAD, *Bacillus subtlis* and *Lactobacillus acidophilus*) were used.

Table (1): Chemical composition of alfalfa hay, concentrate feed mixture (CFM) and mixed ration (on DM basis, %).

Constituents	Alfalfa hay	CFM	Ration
Dry matter (DM)	90.4	89.5	89.8
Organic matter (OM)	87.2	88.3	87.52
Crude protein (CP)	16.4	15.2	16.10
Neutral detergent fiber (NDF)	47.3	40.8	44.7
Acid detergent fiber (ADF)	27.5	13.8	22.02
Gross energy (GE), (MJ/Kg DM)	15.21	16.67	15.79

Experimental design:

An *in vitro* experiment was designed to make a comparison between three groups of probiotics (all probiotics were used in concentration of 2 ml/Kg TMR).
G1 (control): TMR (Alfalfa + CFM).
G2: TMR + ZAD which consisted of [Anaerobic bacteria (*Ruminococcus flavefaciens* ($1x10^9$ /L) + exogenous enzymes (cellulase + xylanase + alfa amylase + protease)].
G3: TMR + *Bacillus subtlis* which consisted of [*Bacillus subtlis* (2×10^{11} CFU) + prebiotic (Mannan oligosaccharides 15.5 g/ L +β-Glucan 17.5 g/L)].
G4: TMR + Probiotic combination which consisted of [ZAD+ *Bacillus subtlis* + *Lactobacillus acidophilus* (1×10^{11} CFU) + Mannan oligosaccharides (15.5 g/ L) +β-Glucan (17.5 g/L)].

In vitro DM and gas production procedure:

In vitro technique was done according to Tilley and Terry (1963) for one stage only (incubation with buffered rumen liquor without incubation with pepsin enzyme) for determination *in vitro* dry matter disappearance (IVDMD%) after different incubation periods till 96h and gas production was measured according to Cappellozza *et al*. (2023) and Makled *et al*. (2019).

Rumen samples were collected from three slaughtered adult Arabian she camels from slaughterhouse near to Cairo. Then rumen samples were immediately transferred in an insulated ice box to the laboratory.

Feed samples (about 0.5g) was incubated with filtered rumen liquor (filtration on four layers of cheese cloths to separate feed particles) and pre warmed buffer (artificial saliva) with (1:4) percent and saturated with CO_2 gas in glass bottle with stopper and crimped with aluminum seals. Incubation was done at 39ºC in water bath for 2, 4, 6,12, 24, 48, 72 and 96h. Three glass bottles were used in each incubation time per group and two bottles as blank to measure gas production and IVDMD% then discarded.

Chemical analysis:

Proximate analysis of feeds including dry matter (DM), organic matter (OM) and crude protein (CP) were determined according to AOAC (1990). Fiber fractions (neutral detergent fiber (NDF) and acid detergent fiber (ADF)) were conducted according to Van Soest and Robertson (1985) using ANKOM Model 220 Fiber Analyser (Macedon, NY, USA). Gross energy was determined using bomb calorimeter (C200, IKA Works Inc., Staufen, Germany).

Calculation:

- *In vitro* dry matter disappearance (IVDMD%)

= [(feed sample weight before incubation) **–** (feed sample weight after incubation **+** blank) / feed sample weight before incubation] x 100. According to Tilley and Terry (1963).

- ME (MJ/kg DM) = 2.20 + 0.136*GP (ml/200 mg DM) **+** 0.057*CP (g/Kg DM)
  OMD% = 14.88 + 0.889 GP (ml/200 mg DM) + 4.5 CP (%) + 0.0651 ash (%) According to Menke and Steingass (1988). Where ME is the metabolizable energy, GP is 24h net gas production (ml/200 mg DM); CP is crude protein (g/Kg DM); and OMD% is organic matter digestibility at 24h of incubation.
- TDN% = [ME (MCal/kg DM) + 0.45] / 0.0445309 (NRC, 1989). Where TDN is total digestible nutrients.
- MPg/ Kg DOM = (19.3 * OMD% *6.25)/100 (Czerkawski, 1986). Where MP is rumen microbial protein
- SCFA (mM)= 0.0239*GP-0.0601

Where SCFA is short chain fatty acid. Output (mM) GP is 24h net gas production (ml/200 mg DM) using the equation described by Getachew et al., (2000).

- GP/gDM= Total gas production (ml) at 24h / substrate DM (g)

GP/gOM = Total gas production (ml) at 24h / substrate OM (g)
GP/gNDF = Total gas production (ml) at 24h / substrate NDF (g)
GP/gADF = Total gas production (ml) at 24h / substrate ADF (g) According to Makled *et al*., (2019).

- The IVDMD characteristics were estimated by using the exponential model proposed by Ørskov and McDonald (1979):

$Y = a + b (1 – e^{-ct})$ where,
Y = the IVDMD% with time (t),
a = soluble fraction (%),
b = insoluble but degradable fraction (%),
e = the natural logarithm,
c = degradation rate of fraction b (%/h) and
(a+b) = potential degradability.
ED (effective degradability) = a + [(b x c)/(c + k)]
K = flow rate assumed to be 0.02 or 0.04 or 0.08
PDMI (Kg/d) = 0.572 + 0.0766*(a + b). According to Ørskov *et al*., (1988). Where PDMI is predicted dry matter intake per day.

Statistical analysis:

Differences among groups were significantly checked using one way analysis of variance (ANOVA) and Duncan's new multiple range test (Duncan, 1955) was used to compare between means. The General Linear Model (GLM) of SAS (1996) was applied. The NLIN procedure of SAS was used to estimate *in vitro* degradation parameters (a,b and c).

RESULTS AND DISCUSSION

Gas production and predicted nutritive value:

Gas production increased gradually with incubation time from 24, 48,72 to 96h as shown in Table (2) and Figure (1). Unfortunately, we couldn't measure gas production in the present study from glass bottles by graduated glass syringe with a needle at 2,4,6 and 12h due to small amount of gas was produced in that time. Also, sensitivity of glass syringes to measure gas volume is may be lower than incubation inside graduated glass syringes (Menke *et al*.,1979) and also may be lower than measuring gas production inside glass bottles by gas pressure transducer sensor (Theodorou *et al*., 1994).

Many researchers noted that gas production from *in vitro* camel rumen liquor was low. Abdel Gawad and Alhadrami (2006) found that gas production (*in vitro* camel rumen liquor) had 0.7 ml/200mg at 3h up to 31ml at 72h of incubation when spartina grass that washed with fresh water was tested. Abdel Gawad (2015) also found the same trend of low gas production produced (at 2, 4 and 6h) from different roughages with camel rumen liquor. Recently, Rabee *et al*., (2022) made a comparison between sheep

and camels as donner of rumen liquor in *in vitro* evaluation of barley straw samples. They recorded gas production at 24, 48 and 72h and found 30.3, 34.7 and 35.7 ml/0.5g DM for camels and 36.0, 44.0 and 44.7 ml/0.5g DM for sheep. However, DMD and NDFD% at 72h were (33.0 and 28.5%) for camels and (34.0 and 28.5 %) for sheep, respectively.

Gas production at 24, 48 and 96h was no significant among all groups (Table 2). However, gas product at 72h was significantly differed in G1(control) and G3 (Bacillus) versus G2 and G4. There was no significant difference between all groups in overall mean followed 35.25, 32.0, 33.13 and 31.50 ml/0.5g for G1, G2, G3 and G4, respectively.

Table (2): Rumen *in vitro* gas production with different probiotics at different incubation times.

Incubation Time, h	Control	Zad	Bacillus	Zad+B+L	±SE	Mean	*P*-Value
24	21.00	19.33	17.00	18.33	0.66	18.92	0.169
48	24.33	22.33	23.33	23.00	1.03	23.25	0.929
72	47.33^{a}	41.00^{b}	44.67^{ab}	41.67^{b}	0.96	43.67	0.037
96	48.33	45.33	47.50	43.00	1.11	46.04	0.355
Mean	35.25	32.00	33.13	31.50	1.81		0.900

a and b means with different superscripts on the same row differ significantly (P<0.05).

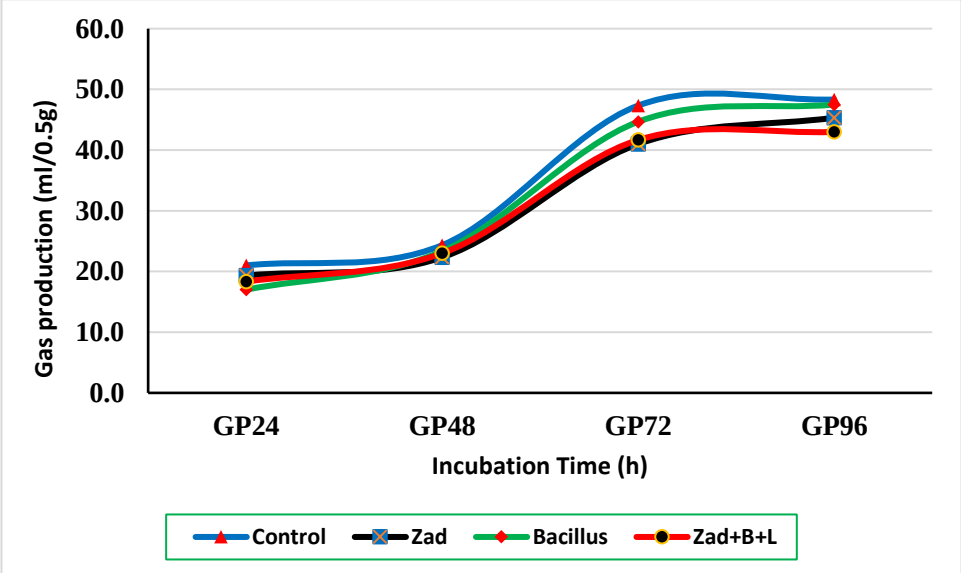

Figure (1): Gas production with different probiotics.

Predicted nutritive values are shown in Table (3). There was no significant difference between groups but there was a slight numerical increase as G1>G2> G4>G3. The values of ME and TDN were 12,65, 12.55, 12.41 and 12.49 MJ/Kg DM and 68.88, 68.34, 67.58 and 68.02% for G1, G2, G3 and G4, respectively. These results reflected the strong correlation between gas production level at 24h (Table 2) and predicted nutritive value (Table 3).

In vitro dry matter disappearance (IVDMD%):

In vitro dry matter disappearance values are shown in Table (4) and Figure (2). The values of IVDMD% ranged from 25.68 to 70.02% through four days (96h) of incubation. There was no significant difference between groups in 2,4 6, 48 and 72h of incubation. The values of IVDMD% at 12 and 24h were significantly differed G3 and G4 versus G1 and G2. At 96h of incubation we found that all probiotic groups G2, G3 and G4 (69.05, 69.05 and 70.02%) were higher (P>0.067) than control group (63.84%), respectively. Also, the overall mean in probiotic groups were higher (but non-significant) than control group. That indicates using probiotic additive with camels can stimulate rumen microorganism which resulted in increasing IVDMD%. Makled *et al*. (2019) found that using probiotic (Lactobacilli isolates with 10^{6}cfu/ kg DM) in in vitro evaluation (alfalfa hay and CFM, R:C= 40:60) increased IVDMD from 43.21% to 46.45% in control and probiotic groups, respectively.

Table (3): Predicted nutritive value with different probiotics.

Item	Control	Zad	Bacillus	Zad+B+L	±SE	*P*-Value
ME, (MJ/Kg DM)	12.65	12.55	12.41	12.49	0.04	0.175
ME/GE	0.803	0.797	0.787	0.793	0.01	0.136
TDN%	68.88	68.34	67.58	68.02	0.21	0.168
SCFA mM /g DM	0.813	0.727	0.607	0.673	0.03	0.174
MP (g/Kg DOM)	37.51	36.71	35.60	36.24	0.31	0.169
GP/gDM, ml	46.66	42.96	37.78	40.74	1.50	0.169
GP/gOM, ml	53.34	49.11	43.18	46.57	1.67	0.169
GP/gNDF, ml	104.37	96.09	84.49	91.12	1.55	0.337
GP/gADF, ml	211.89	195.10	171.53	184.98	6.63	0.169

ME: Metabolizable energy, GE: Gross energy, TDN: Total digestible nutrients, SCFA: Short chain fatty acids, MP: Microbial protein, DOM: Digested organic matter, GP: Gas production at 24h, DM: Dry matter, OM: Organic matter, NDF: Neutral detergent fiber and ADF: Acid detergent fiber.

Table (4): *In vitro* dry matter disappearance % with different probiotics at different incubation times

Incubation (Time, h)	Control	Zad	Bacillus	Zad+B+L	±SE	Mean	*P*-Value
INV2	25.68	25.69	25.610	27.26	0.41	26.06	0.474
INV4	27.29	28.36	27.03	28.02	0.25	27.68	0.226
INV6	38.37	38.56	38.57	37.88	0.33	38.34	0.906
INV12	39.68^{b}	39.63^{b}	43.33^{a}	42.88^{a}	0.57	41.38	0.002
INV24	60.14^{b}	60.59^{b}	62.077^{a}	62.083^{a}	0.37	61.22	0.013
INV48	61.580	60.623	62.260	62.373	0.36	61.71	0.364
INV72	62.12	63.58	63.38	64.44	0.35	63.38	0.106
INV96	63.84	69.05	69.05	70.02	0.97	67.99	0.067
Mean	47.34	48.26	48.91	49.37	1.64		0.976

a and b means with different superscripts on the same row differ significantly (P<0.05).

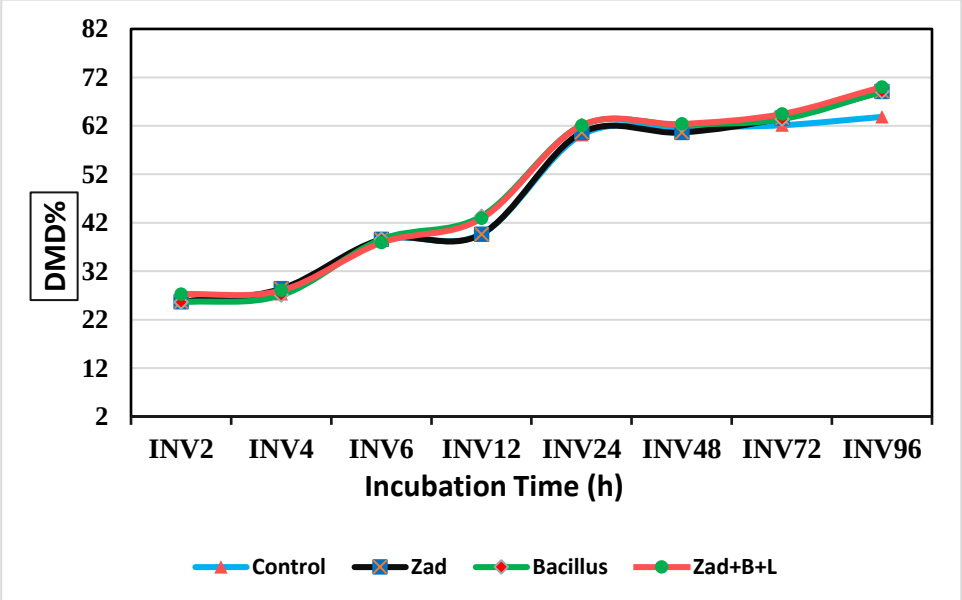

Figure (2): *In vitro* dry matter disappearance with different probiotics

On the other hand, camels take time for increasing degradation of DMD%, Jouany *et al*. (1995) made a comparison between sheep and camels in *in situ* degradation of DM% for alfalfa and they found 21.8

and 19.9% (P>0.05) for 6h and 49.4 and 53.7% (P<0.05) for alfalfa DMD% at 72h for sheep and camels, respectively. And this is associated with increasing solid particle retention time in camels than sheep.

In vitro degradability characteristics:

Dry matter Degradability values and predicted dry matter intake (PDMI) are shown in table (5). The soluble fraction (a) in Zad group (G2) was 20.16% with a significant difference compared to the other experimental groups. May be the presence of exogenous enzymes (cellulase + xylanase + alfa amylase + protease) in Zad group enhanced and increased the soluble fraction (a). The same effect was noted by Yang *et al*., (2022) on whole plant faba bean silage that treated with gradually levels of exogenous fibrolytic enzyme derived from *Trichoderma reesei* with concentrations from zero to 1.5 (ml/Kg) that resulted in increasing soluble fraction of DM from 25.10 to 29.24%, respectively. Using exogenous fibrolytic enzymes resulted in pre-digestion effect that increased the soluble fraction of DM (Pinos-Rodrıguez *et al*., 2008).

Table (5): In vitro DM degradability characteristics.

Item	Control	Zad	Bacillus	Zad+B+L	±SE	Mean	*P*-Value
a% (Instant degradable)	18.60^{c}	20.16^{a}	17.39^{d}	19.69^{b}	0.32	18.96	<.0001
b% (Insoluble but potentially degradable)	44.97 ^{d}	45.84^{c}	47.31^{b}	48.58^{a}	0.42	46.68	<.0001
(a+b) % (Potential degradability)	63.56^{c}	65.99^{b}	65.97^{b}	67.00^{a}	0.38	65.63	<.0001
C% /h (Degradation rate)	0.07^{b}	0.06^{c}	0.07^{b}	0.08^{a}	0.002	0.07	<.0001
ED (0.02)	53.89^{d}	54.93^{c}	55.94^{b}	56.25^{a}	0.28	55.25	<.0001
ED (0.04)	47.63^{d}	48.16^{c}	49.34^{b}	49.48^{a}	0.24	48.65	<.0001
ED (0.08)	40.04^{d}	40.32^{c}	41.19^{b}	41.43^{a}	0.18	40.75	<.0001
Intake (Kg/d)	5.44^{c}	5.63^{b}	5.63^{b}	5.71^{a}	0.03	5.60	<.0001

a, b, c and d means with different superscripts on the same row differ significantly (P<0.05).

The b, (a+b), c, effective degradability values (E0.02, E0.04 and E.0.8) and predicted DMI (PDMI) values (Table 5) were significantly differed, and the highest values were found in G4 (mixture of probiotics) that consisted of probiotic combination (*Ruminococcus flavefaciens* and exogenous enzymes, *Bacillus subtlis* + prebiotics and *Lactobacillus acidophilus* and prebiotics) which maybe had positive synergistic effect between these different probiotics. This can be explained by higher values of IVDMD% of G4 in the most of incubation time from 2 to 96h and higher on over all mean of IVDMD (49.37%) than the other groups (Table 4).

In the same way, Pan *et al*. (2022) reported that there was a positive synergistic effect when using *Bacillus spp* (*B. subtilis* and *B. licheniformis*) as probiotic (3.2×10^{9} CFU per g) which reflected on significantly improving IVDMD% for roughage (mostly at 48h) and concentrate (mostly at 24) in separate incubation. And this maybe done due to production of expansin-like proteins from *B. subtilis* that improve and increase efficiency of *B. licheniformis* fibrolytic enzymes (cellulase) by expanding or disrupting plant cell wall components (cellulose and hemicellulose). Same results were found by Cappellozza *et al*. (2023) using *Bacillus spp* (*B. subtilis* and *B. licheniformis*).

Additionally, using lactic acid bacteria (*Lactobacillus plantarum*) as probiotic (*in vitro*) significantly increase rumen total bacteria and fibrolytic bacteria (*Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens*), total protozoa and OMD% (Izuddin *et al*., 2018). Predicted DMI (PDMI) was significantly higher in G4 (5.71 kg/d) than the other groups (Table 5) due to a positive synergistic effect of mixture of probiotics in G4 that resulted that the highest values in IVDMD% (Table 4), a+b, c and effective degradability (Table 5) were belonging to G4, so it had the highest predicted DMI.

Even though the equation for prediction of DMI (Kg/d) was computed by Ørskov *et al*. (1988) for cow but it can be used for camel (Table 5) just as indicator of *in vivo* situation. Assuming camels with 400 Kg and according to the present results the feed intake as a percent of body weight will be 1.36, 1.41, 1.41 and 1.44% for G1, G2, G3 and G4, respectively. These results were closely related to the results of Farid *et al*. (1990) who recommended 1.24% DMI (as percent of BW) as maintenance of DMI for 400 Kg camel.

CONCLUSION

It can be concluded that using probiotics in combination (ZAD, *Bacillus subtlis* and *Lactobacillus acidophilus*) in *in vitro* (using camel's rumen liquor) induced significant positively synergistic effects such as enhancing DM digestibility, degradability parameters and predicted dry matter intake. Future *in vivo* studies are needed to confirm these results.

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تأثير استخدام البروبيوتك فى تغذية الابل العربية: دراسة معملية

أمل مصطفى عرفة[1,2] وهانى جادو[1] و مصطفى محمد غندور[2] و حمدى موسى متولى[1] و احمد رجب عسكر[2]

[1]قسم الانتاج الحيوانى، كلية الزراعة – جامعة عين شمس – شبرا الخيمة – القاهرة - مصر

[2]قسم تغذية الحيوان و الدواجن – مركز بحوث الصحراء – المطرية - القاهرة - مصر

تهدف هذه الدراسة لمقارنة استخدام عدة انواع من البروبيوتك (منفردة او مختلطة) معمليا. فتم تحضين عليقة من دريس البرسيم الحجازى و العلف المركز بنسبة (40:60) معمليا مع سائل كرش الابل حتى زمن 96 ساعة و قسمت المجموعات الى مجموعة المقارنة (G1) و مجموعة (G2, ZAD) و مجموعة (G3, Bacillus) و مجموعة مختلطة من البروبيوتك (G4, ZAD+ *Bacillus*+ *Lactobacillus*). و تم استخدام البروبيوتك بتركيز(2 ميللميتر^3/كجم مادة جافة). و تلخصت اهم النتائج فى الاتى: لم تكن هناك اختلافات معنوية فى متوسط انتاج الغاز معمليا بين المجموعات. ادى استخدام البروبيوتك بأنواعه المختلفة لتحسين هضم المادة الجافة مقارنة بمجموعة المقارنة (على متوسط الازمنة و المتوسط العام). وادى استخدام مخلوط البروبيوتك لحدوث تأثير تآزورى موجب بين مكونات المخلوط مما ادى لتحسن معنوى فى الجزء القابل للهضم و معدل الهضم للمادة الجافة و كذلك تحسن فى قيمة المأكول (المتنبأ به).