Ankom Daisy^{II} modifications to stabilise the rotation speed

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Abstract— **Ankom Daisy^{II} incubator (Ankom Technology, Macedon NY, USA) has gained acceptance as an alternative to traditional *in vitro* procedures to measure feed degradability. It reduces labour requirements and increases the number of determinations that can be completed by a single operator. The apparatus allows simultaneous incubation of multiple feedstuffs in the same jar, weighed in single bags, and placed in a continuously rotating incubator at 39.5 °C with a buffered *inoculum*. This simple procedure shows some functional limitation that can be resolved to obtain more reliable results. In particular, the jars do not rotate smoothly, with slowdowns, stops, and restarts that increase the variability of the instrument itself. In this paper, some low-cost and easily implementable structural adjustments are proposed, modifying the two drive rollers, and using a new drive pulley. These modifications can ensure steady conditions which are necessary in scientific experiments and reduce the doubts of the variability in degradability results related to an inaccurate instrumentation functionality.**

Keywords— Ankom Daisy^{II}, modifications; constant rotation; feed degradation reliability.

I. Introduction

In animal nutrition, the evaluation of degradability of feeds is a very important issue, which many researches have focused on, using techniques ranging from *in vivo* to *in vitro* methods for both ruminants and monogastrics.

In vitro methods simulate digestion processes in the laboratory and offer the advantage of being more precise, rapid, and less labor-intensive than *in vivo* methods, not requiring the presence of animals and being able to be applied simultaneously for different feeds. Daisy^{II} incubator (AD^{II}, Ankom Technology, Macedon NY, USA) allows simultaneous incubations of different feedstuffs in sealed polyester bags, and placed in the same incubation jar with a buffered *inoculum* (rumen fluid, or feces, or enzymes).

The AD^{II} eliminate the laborious work involved in the conventional procedures such as the requirement for a high number of test tubes and filtering samples after degradation. Instead, it simply uses four digestion jars, each capable of containing up to 24 feed samples while maintaining the anaerobic conditions at the temperature of 39.5 °C and a shaking movement to ensure microbial activity, as a necessary condition for a proper incubation procedure [1].

Several authors [2,3] verified that, for some feedstuffs, the degradability values provided by AD^{II} incubator were not statistically different from the results obtained with the tube procedure [4]. Similarly, Lattimer, Cooper, Freeman, and Lalman [5] showed that the use of equine fecal *inoculum* in the closed-system fermentation apparatus (AD^{II}) gave a correct estimation of DM, NDF and ADF *in vitro* digestibility of high-quality diets.

The AD^{II} should ensure a continuous and regular agitation for several hours, according to operational requirements. Generally, the continuous period of agitation/rotation of the jars, containing the bags and the liquid, varies from 24 to 72 hours [6]. However, the AD^{II} agitation mechanism has reliability problems [7]. Our experience has revealed a variability which is difficult to predict in its erraticity, especially over long periods of time (72 hours).

Even if AD^{II} is fully functional, the jars are not rotating smoothly, with slowdowns, stops and restarts. This is a common and frequent problem, so much so that on the Ankom web site [8] it is also reported that "*The jars are not rotating well*" and suggests solutions.

Despite the attention paid and the application of the suggestions proposed by the Ankom [8], the problem has not been solved until now. Therefore, the inherent variability of the instrument is increased, the standard conditions of analysis are not assured, and the degradability measurements are partially influenced [7]. This has led us to look for a solution to ensure more regular rotation of the AD^{II} incubator.

The objective of this work is to propose and describe some simple and inexpensive structural modifications aimed at regulating the rotation of the AD^{II} jars, thereby minimizing the unpredictable and erratic source of functional variability.

II. MATERIALS AND METHODS

Two AD^{II} incubators (AD1, AD2) located in two different laboratories, where incubators have been using for many years to study feed degradability [9,10,11,12], were employed to test modifications.

The AD^{II} incubator (Figure 1) consists in a thermostatic chamber with four rotating digestion jars that contain: samples weighed in filter bags (F57, Ankom Technology, Macedon NY, USA)**,** buffer solution and *inoculum*, as described by Tassone et al. [10].

FIGURE 1. The Daisy^{II} incubator (Ankom Technology Corporation Fairport, Macedon, NY, USA).

The rotation system is made up of an electric motor, with a drive pulley, placed in the center of the four jars, connected by two drive belts to two drive rollers. One drive roller is placed between the two upper jars and the other drive roller is positioned between the two lower jars. Each pair of jars is supported by its respective rotating drive roller and three additional free rollers. The rotation of the upper drive roller results in simultaneous movement of the upper pair of jars (identified from left to right as 1 and 2), while the rotation of the lower drive roller causes the lower pair of jars (identified from left to right as 4 and 3) to move simultaneously.

Different modifications and solutions were tested on AD1 to find the optimal combination to assure a constant rotation of the jars and then they were applied also to AD2. The modifications included the adjustments of pulley, drive belts, and rollers.

Each AD^{II} was tested by measuring the consistency of the rotation in both the original or modified versions, with the jars empty or filled with 2 L of liquid.

The consistency of the rotation was also tested during degradation tests, with rumen fluid (RF) and sample bags. In this case, the instruments were adapted, allowing pairs of jars to work simultaneously in the original and modified version alternately (upper pair original version *vs* lower pair modified version and vice versa). This was done to reduce the effect of rumen fluid variability on degradability results. In any feed degradability analysis, the rumen liquid was collected in a slaughterhouse, immediately transported to the laboratory, and placed in jars after appropriate preparation.

To detect the rotation speed (round/hour) of each jar, a rev counter data logger was specially designed and assembled (Figure 2a). A magnet was positioned on the side of the lid of each jar (Figure 2b) and four Hall effect sensors (Figure 2c) were placed on a specially designed support inside the AD^{II} (Figure 3) to detect the magnetic field.

A software has been implemented on Arduino® (Figure 2d) and provided to save the time of each passage for each magnet on a SD memory card (Figure 2e). The rotation speed detection was carried out for a minimum of 48 hours with the AD^{II} maintained at 39.5 °C. The check has been done for each of the four jars and the comparison between the jars was performed individually, although each pair of jar, upper and lower, was moved by a common drive belt.

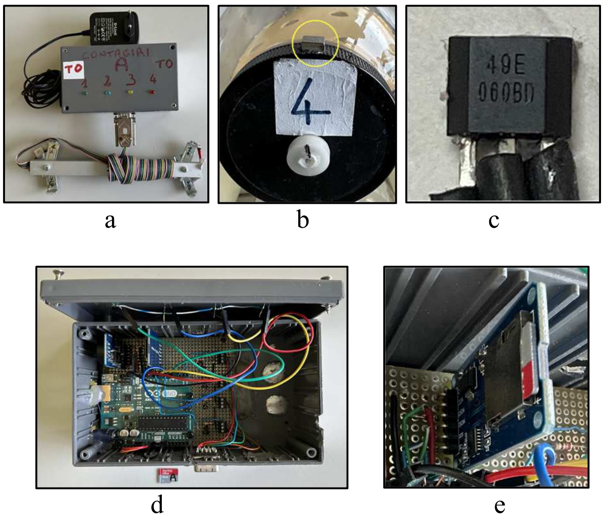


FIGURE 2. Rev counter data logger: a - complete data logger; b – magnet glued to the edge of the jar cap; c – Hall effect sensor; d – micro-SD memory card to record data; e - Arduino® card.

Results of degradability measurements were reported by Tassone, Sarnataro, Glorio Patrucco, Mabrouki, and Barbera [7].

The detected parameters were: the rotation speed expressed as number of rounds per hour (rph); the delay, expressed as speed loss in percent compared to the rotational speed of the empty jar in the two original and modified versions.

$$delay_{ij}\ \% = \frac{erph_{ij} - rph_{ij}}{erph_{ij}} \times 100$$

where:

- $erph_{ij}$ = round per hour for the empty jar_{ij};
- rph_{ij} = round per hour for the filled jar_{ij};
- i = 1 is the original and 2 the modified instrument;
- j = 1 to 4 indicating the position of the jar.

The delay parameter which measures the percentage speed loss was introduced to consider the different speeds due to the two drive rollers with slightly different diameters. The engraving of the tracks was not done in a perfectly even way. A minimal difference in diameters translates into a different average hourly rotation speed which could alter the analysis of the results. Furthermore, during the experimentation, the two drive rollers were assembled and disassembled several times to switch from the original version to the modified version and vice versa. The same component was not always

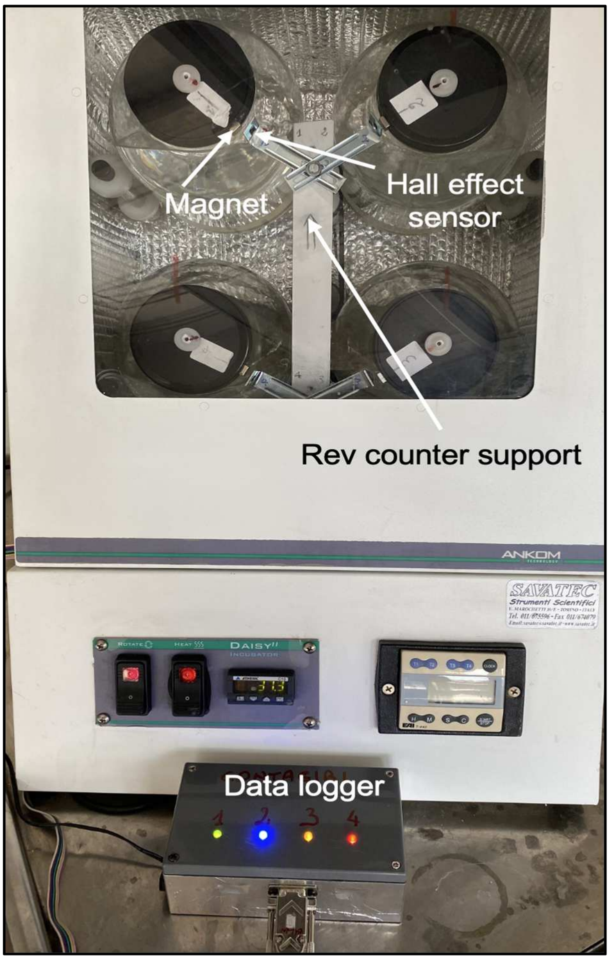


FIGURE 3. Rev counter data logger installed on the Daisy[II] incubator.

reassembled in the same position.

Data were processed, with SAS [13], and a GLM procedure was used to evaluate the effects of the modification (original *vs* modified AD[II]).

III. RESULTS AND DISCUSSION

The results describe in detail the modifications made to the AD[II] to ensure a regular and simultaneously rotation of all jars, at the same speed and without stops.

There are two main problems that cause malfunctions on the AD[II]:

a) the sliding of the drive belts on the drive pulley and on the drive rollers, which causes a reduction in rotation speed;

b) the sliding of the jars on the free rollers that do not rotate freely due to the weight of the jars themselves, which induces a further slowdown.

Based on these two aspects, AD[II] was adjusted by modifying or creating new components.

a) Sliding of the belts and drive belts

To solve this problem two belts and two drive belts for each pair of jars (upper and lower pair) were used and therefore the relative drive pulleys and drive rollers were modified.

A new drive pulley, using a piece of polycarbonate with four tracks to accommodate four drive belts, has been created as shown in Figure 4. Polycarbonate was chosen as the material because the inner surface of the tracks retains a rough surface, making it more suitable for the application. To block the new drive pulley to the engine, a 4 mm screw was positioned after practicing a 3.2 mm hole and filled at 4 mm, as in Figure 4.

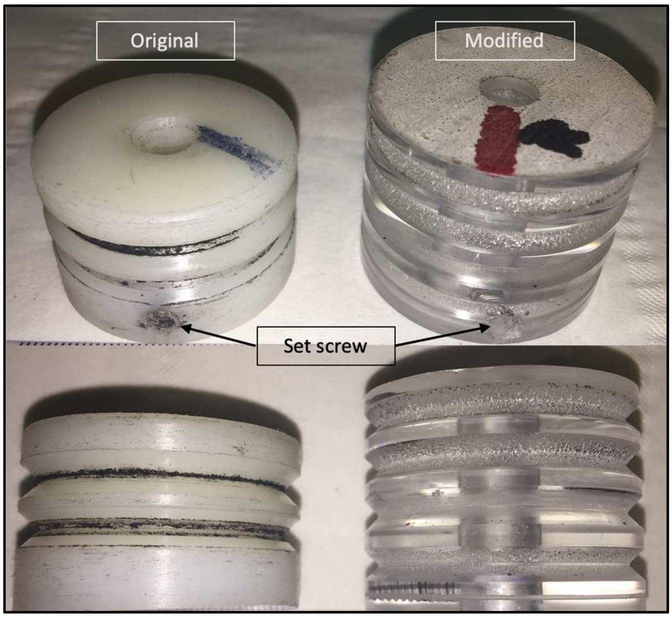


FIGURE 4. Original and modified drive pulleys.

To obtain two drive rollers to fit two drive belts each, the two original drive rollers, included with AD[II], were modified. Into the original drive roller, two additional tracks were carved as shown in Figure 5, to be able to position the two additional drive belts.

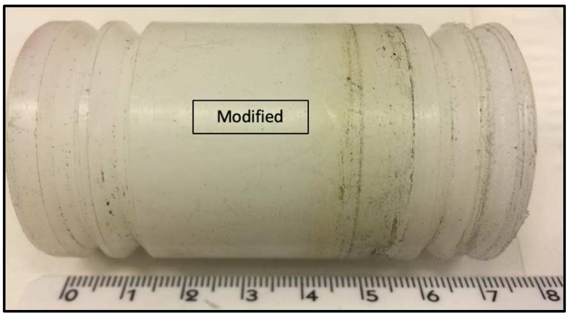


FIGURE 5. Modified drive roller.

It is important to position the two drive belts that control the lower pair of jars on the two rearmost tracks of the modified drive roller and drive pulley to prevent them from rubbing against the bottom of the lower jars (Figure 6).

Figure 7 summarizes the technical drawings with the dimensions of the modified drive pulley and drive roller to reproduce them and adapt to AD[II].

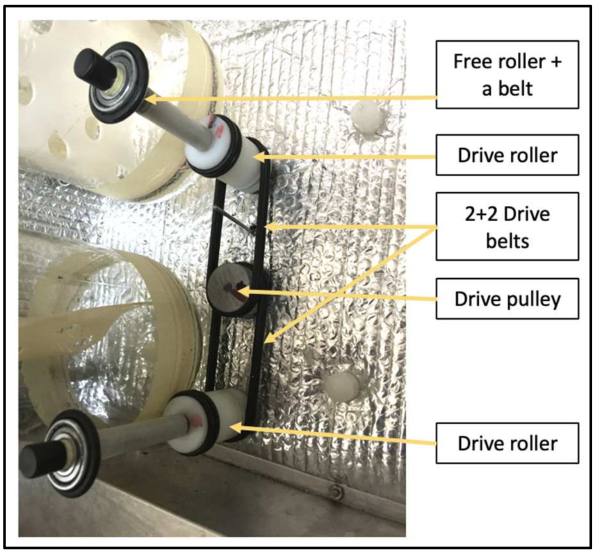


FIGURE 6. Correct positioning of belts and drive belts.

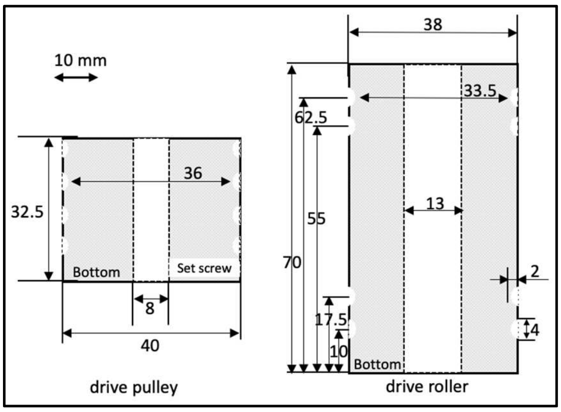


FIGURE 7. Technical drawings with the measurements of the pieces made or modified.

b) Sliding of the jars

The weight of the four jars containing the bags, and the movement of the bags and the ruminal liquid inside the jars cause an increase in friction between the jars and the free pulleys and supporting pins, onto which they are inserted. This means that, even though the drive belts run correctly, the jars turn slowly or even stop, and then start again in a completely random way. The same thing arises between the jars and the drive rollers, which also slide due to the weight of the jars themselves, making the rotation of the four jars irregular.

The solutions proposed by Ankom on its website [8] to the problem of poorly rotating jars are:

1. "*There is not enough friction between the outside surface of the jars and the rollers on the instrument. This is … find the tape that athletic trainers use to tape athletes works best.*
2. *The drive belts may be slipping. This may occur if the Daisy has a bit of age on it. The drive belts … Remove the o-ring from one pulley and twist the o-ring into a figure 8. This will cause the o-ring to "shorten" and will keep it tight on the pulleys.*

Our suggestion to avoid the slowing down or stopping of rotation due to the weight is to replace the 10 original free rollers by ball bearings with belts. Ball bearings (ext ∅ = 35 mm; width = 15 mm; int ∅ = 13 mm) are available on the market at low price that are well suited to replace the original free rollers. The internal diameter of 13 mm is larger, but it is sufficient to place a paper cylinder as an adapter, as in Figure 8.

Moreover, the original belts fit the ball bearings. However, to ensure that the belts stay securely in place during use, a track must be engraved on the outside of the ball bearing or a hard glue to place the belt. In this way, it is possible to prevent belts from shifting during rotation.

The addition of the belts to the ball bearings significantly increases the friction with the jars and further stabilises their rotation. Also, the second belt on the drive roller helps to maintain constant the rotation of the jars.

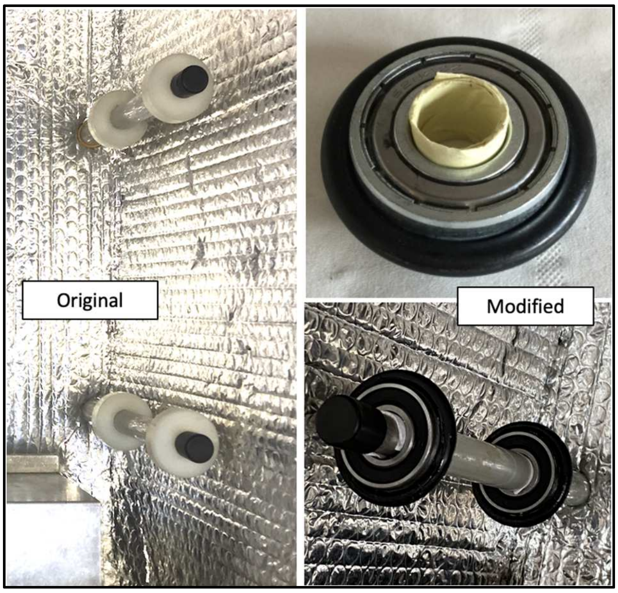


FIGURE 8. Ball bearings with belts replacing free rollers.

Data analysis

To verify the effectiveness of the introduced changes, two parameters were analysed: rounds per hour (rph) and the delay in percent of each jar filled with liquid or rumen fluid and bags with respect to the corresponding empty jar. This highlights the relationship between the problem and the increased weight of the jars themselves.

Table 1 shows the mean values of speed and delay rotation by jar and significance of the comparisons relating to the two pairs of jars (1 & 2 *vs* 3 & 4) as each pair is moved by on (original) or one pair (modified) of belts. When ruminal fluid and bags were used, only one jar for pair was filled with. The second one, for pair, was filled with water to maintain the constant load.

Although all comparisons between upper and lower pairs of jars are always significantly different, it is interesting to observe the relative values. Firstly, the differences in absolute value between pairs of jars, as described before, are due to the structural differences in the diameters of the original and

TABLE 1. Speed and delay (% of rph less than empty jars) LSMEANs of jars according the original or modified Daisy[II] after 48 hours.

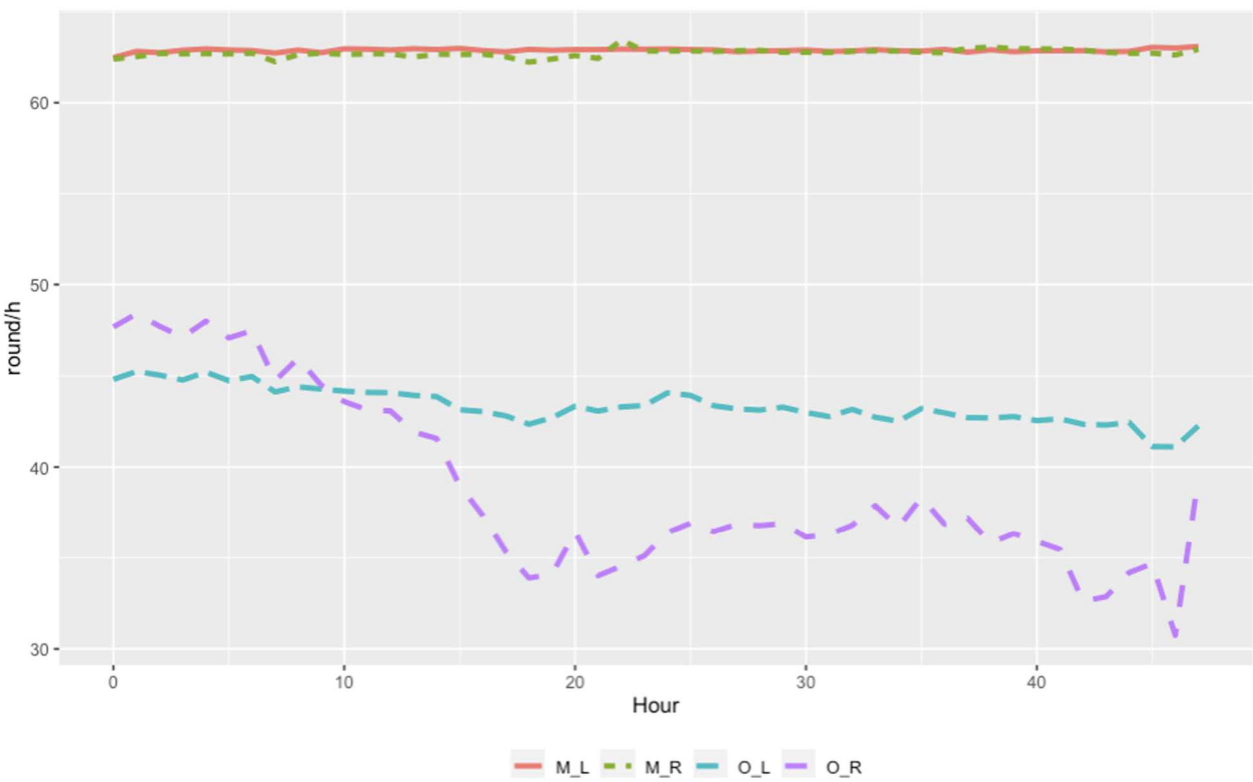
Parameter	Content of jars	System	Jar1	Jar2	Jar3	Jar4	Jars 1 & 2 *vs* 3 & 4	MSE	DFE	P
Speed (rph)	Empty	Original	55.3	55.2	56.3	56.0	P < 0.0001	7.95	128976	< 0.0001
		Modified	61.4	61.1	63.9	63.8	P < 0.0001			
	Liquid	Original	30.0	39.1	48.2	45.2	P < 0.0001	31.90	152053	< 0.0001
		Modified	61.8	62.2	63.4	63.4	P < 0.0001			
	Rumen fluid + bags	Original	46.2	-	32.6	-	P < 0.0001	34.85	23687	< 0.0001
		Modified	60.9	-	64.0	-	P < 0.0001			
Delay (%)	Liquid	Original	46.1	26.6	13.2	18.7	P < 0.0001	86.83	152053	< 0.0001
		Modified	-0.2	-1.9	1.1	1.4	P < 0.0001			
	Rumen fluid + bags	Original	14.3	-	42.6	-	P < 0.0001	102.14	23687	< 0.0001
		Modified	-1.9	-	1.2	-	P < 0.0001			
CV (rph)	Empty	Original	4.5	4.6	3.8	3.8				
		Modified	5.2	4.8	4.8	4.9				
	Liquid	Original	29.6	27.7	5.41	17.3				
		Modified	7.0	7.2	6.1	6.0				
	Rumen fluid + bags	Original	23.6	-	27.4	-				
		Modified	5.9	-	6.0	-				

CV: coefficient of variation. LSMeans difference within each row.

modified drive rollers and drive pulleys, which determines different rph values.

Observing the Table 1, it is evident how the speeds of the jars slow down considerably in the original instrument with the jars filled with liquid or ruminal fluid. Figure 9 shows the average rotation speed for the version (original or modified) and the content (liquid or rumen fluid with bags). The original version has a general slowdown which is accentuated by the presence of the bags. Inside the jar there is a dividing septum with the aim of keeping the bags immersed in the rumen liquid, but this causes a variable resistance to the rotation which increases the irregularity of the instrument. In the modified version, the rotation speed per hour remains very stable over time.

FIGURE 9. Average rotation speed (rph) by instrument version and content
(O_L = original & liquid; O_R = original & rumen fluid with bags; M_L = modified & liquid; M_R = modified & rumen fluid with bags).



The rotation speeds of the empty jars differ slightly between the original and modified Daisy^{II} due to small variations in the diameters of the drive rollers and pulleys. In any case, the variability of the rotation speed of each empty jars was very small in both versions, oscillating between 3.8 and 5.2 %. The delay results will be discussed at place of the rotation speed to eliminate this structural bias.

The average delay of the two versions, original and modified, was significantly different both for liquids (23.3 *vs* 0.2 % respectively) and for rumen fluid plus bags (25.8 *vs* 0.0 % respectively).

The instrument not only experiences slowdowns but also results in complete stoppages, causing the jars to remain motionless for extended periods, which in turn leads to an increase in variability. When functioning with empty jars, both the original and the modified AD^{II}s have an overall average delay standard deviation of 3.3 and 2.4 rph respectively. With jars filled with liquid or rumen fluid, the original AD^{II} has an average standard deviation of 10.0 and 12.2 rph, respectively. The modified AD^{II} has a slight increase in the average standard deviation equal to 4.2 and 4.0 rph, respectively.

Even though the rotation mechanism is working per pair, upper or lower, the jars have different delays (Table 1). In the modified version, the delay for each jar (liquid or RF), although significantly different amongs jars, vary only between -1.9 and 1.4%. In the original version the delay for jar (liquid or RF), shows a wiser difference ranging from 14.3 and 46.1 %. The jars filled with liquid demonstrated that the delay of jar 1 (46.1 %) was much higher than jar 2 (26.6 %), although the movement is controlled by the same drive belt. If we compare it with the other pair, we note a smaller average slowdown of jar 3 and 4, but always different from each other, contributing to making the instrument even more unpredictable.

The results obtained show a substantial improvement by the modified instrument with a stabilization of jar rotation over time. This improvement, by reducing the instrumental variability, will help to correctly study degradability of feeds.

IV. CONCLUSION

The AD^{II} is an instrument designed to work for a long period of time, even up to 72 hours, therefore operational constancy is certainly essential for reliable analytical results. The variability of the rotation speed could be an issue and the suggestions made by the producer do not fully resolve the problem. In this paper, simple changes are proposed and described to solve the rotational problems of the AD^{II} incubator.

The doubling of the transmission belts increased the power transmitted to the jars by reducing the skidding of the drive belts themselves. Moreover, the elimination of unnecessary friction which contributed to slow down the free rolling of the jars was succeeded.

The manual skill required to make the proposed modifications is relatively limited and any craftsman can make the new components or modify and adapt the original components.

Preparing a new drive pulley and modifying the two drive rollers, according to the indications given in this paper the efficiency of the AD^{II} can be improved. This can assure regular and continuous rotation of the jars over time and a reliable measure of the feed degradability [7], allowing for correct experimentation and laboratory activities.

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