The effect of *Saccharomyces cerevisiae* on ruminal fermentation in dairy cows

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ABSTRACT: This study presents the results of an experiment in which the effect of addition of a yeast culture (*Saccharomyces cerevisiae*, Strain 47) on rumen fermentation was studied in thirty-six dairy cows of Holstein breed. The animals were divided into one control and five experimental groups. Each group involved 6 individuals. The animals received a diet consisting of good maize silage with a higher dry matter content (16 kg), clover-grass haylage (l6 kg), meadow hay (3 kg) and supplementary feed mixture (7.5 kg). The rations were fed to cows as total mixed ration (TMR). In experimental groups, the yeast culture was added into the feed mixture in amounts of 2, 4, 6, 8, 10 g per day and animal. Samples of rumen fluid were taken perorally 3–4 hours after feeding. The obtained results indicated that the addition of a *Saccharomyces cerevisiae* SC-47 culture in recommended doses showed a positive effect on ruminal digestion. As compared with control, the addition of all aforementioned amounts of the yeast culture into the feeding ration resulted in all cases in a statistically significant ($P < 0.01$) decrease in pH and fluctuated near the lower limit of the reference values. As compared with control, the yeast culture supplementation showed a positive effect ($P < 0.01$) on production of volatile fatty acids (VFA) (127.6 vs. 84.0 mmol/l). The utilisation of ammonia was higher ($P < 0.01$) in experimental groups (8.12, resp. 8.68 mmol/l) than in controls (9.06 mmol/l). The difference in protozoa numbers in rumens of dairy cows in the control and experimental groups was statistically highly significantly ($P < 0.01$) different. There was a close relationship between the dose of yeast culture on the one hand and the VFA content and protozoa numbers on the other. The regression analysis of dependence of dependent variable (i.e. pH of rumen fluid) on the independent one (i.e. the dose of yeast culture) revealed only a slight degree of dependence ($r = 0.671$).

Keywords: dairy cows; *Saccharomyces cerevisiae* culture; rumen fluid; *Infusoria*; rumen fermentation

In the Czech Republic yeast cultures have recently become an occasional component of feeding rations for cattle. Some papers informed about significant effects of yeast cultures on fermentation under specific anaerobic conditions existing in rumen. Numerous studies (Sune et al., 1998; Alshaikh et al., 2002; Lila et al., 2004 and others) documented positive effects of yeast cultures not only on the rumen environment of dairy cows but also on the improvement of microbial activities. According to Blake (1993), yeast cultures obviously improve the cellulolytic activities of rumen microorganisms in such a way that they increase their total numbers, improve fibre digestion, reduce lactate accumulation, reduce the concentration of oxygen in rumen fluid and improve utilisation of starch supplied in the feeding ration. In this way they influence (inhibit) the rate of VFA production and, thus, increase the stability of rumen environment and improve the intensity of digestion. Sullivan and Martin (1999) also reported that the supplement of a *Saccharomyces cerevisiae* yeast culture into the diet of dairy cows improved the utilisation of lactate and digestion of cellulose. Lyons (1993) and Strohlein (2003) stated that some yeast strains showed a better capability to use lactate because they stimulated its utilization by propionic acid bacteria. The utilisation of lactate by these bacte-

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ria is of the major importance for the stabilisation of rumen environment. Doreau and Jouany (1998) observed that in individual animals yeasts reduced daily fluctuations in pH values and also decreased differences existing between them. This resulted in a higher stability of rumen environment during the day. The effect of different doses of yeast culture *Saccharomyces cerevisiae,* strain SC-47 (0, 3, 6 and 12 g of yeast/day respectively) on the lactating performance of Holstein dairy cows was described by Nikkhah et al. (2004). They drew a conclusion that the yeast culture had a beneficial effect on the rumen health. Other available data indicated that in the rumen fluid of animals receiving supplements of yeast culture the total content of VFA and the percentage of propionic acid (Sullivan and Martin, 1999) and acetic acid (Nursoy and Baytok, 2003) increased, the content of ammonia decreased (Enjalbert et al., 1999; Kamra et al., 2002; Nursoy and Baytok, 2003; Strohlein, 2003) and the total numbers of ruminal bacteria and infusoria significantly increased (Sune, 1998; Kamra et al., 2002 and others). Doreau and Jouany (1998), Zhang et al. (2000) and Strohlein (2003) also reported that the addition of *Saccharomyces cerevisiae* yeast into the feeding ration of dairy cows improved their milk performance significantly. A positive effect of yeasts on the performance of dairy cows and on the content of milk components resulted from increased daily feed intake and improved digestibility of nutrients (Erasmus et al., 1992; Jouany, 2001). In their experiment Nikkhah et al. (2004) did not find the dry matter intake and milk yield in cows to be affected ($P > 0.05$) by experimental diets but milk composition including fat and percent total solids were improved by the addition of yeast culture ($P < 0.05$). In experiments of Biricik and Yavuz (2001) no significant differences were observed in rumen fluid pH, total volatile fatty acid and ammonium nitrogen levels between control and experimental cows. Mruthunjaya et al. (2003) stated that dietary supplementation of Yea-sacc^{1026} (yeast culture of *Saccharomyces cerevisiae,* strain 1026) at the dose of 10 g/cow/day did not influence the whole tract digestibility of nutrients in cows. A significant increase in degradability of roughage in 6 h ($P < 0.05$) after dried yeast addition was described by Ando et al. (2004). The effect of direct-fed microbial supplementation on the performance of dairy cows during the transition period was studied by Nocek et al. (2003). During the postpartum period, dry matter intake, milk yield, and milk protein content were higher in cows receiving direct-fed microbial supplementation compared with the control group. Effects of different doses and rations of roughage and concentrates on the milk performance and efficiency of rumen digestion were discussed also by Hoover et al. (1986), Strzetelski et al. (1996) and others.

The objective of this study was to evaluate the effect of different doses of *Saccharomyces cerevisiae* yeasts (strain SC-47) on the rumen fermentation of dairy cows in the first third of lactation.

MATERIAL AND METHODS

An experiment was conducted to study and evaluate the effects of increasing doses (0, 2, 4, 6, 8 and 10 g/head/day) of the Biosaf yeast culture containing *Saccharomyces cerevisiae* strain SC-47 (Ekozym Ltd., Vizovice, Czech Republic) on biochemical parameters of rumen fermentation in dairy cows. The Biosaf yeast culture used in this experiment contains as the effective agent living non-pathogenic yeasts of the *Saccharomyces cerevisiae* species, strain SC-47 (NCYC) in the minimum amount of 8×10^9 CFU/1 g ($\pm$ 0.27×10^{10}). The product contains 38–45.5% of N-substances per kg of DM and shows a high level of thermostability (from –80°C to +70°C).

The experiment involved six groups of dairy cows ($n = 6$) with similar milk production (30–35 l) in the first third of lactation, viz. one control (0 g) and five (2, 4, 6, 8 and 10 g) experimental groups. All animals received the same feeding ration either without (control) or with the yeast culture supplement. Different doses of yeast culture were mixed into the premix of production feed mixtures. The feeding ration was based on maize silage with an increased DM content (16 kg), clover-grass haylage (16 kg), meadow hay of average quality (3 kg) and concentrate (7.5 kg). The experiment lasted 60 days. The sampling of rumen fluid was performed after 50 days of feeding the ration containing the yeast culture. Samples were taken perorally directly in the barn using a probe that was connected with a low-pressure manual pump. Samples of rumen fluid were cooled in a special bag containing ice and transported to the laboratory where they were filtered through a gauze layer. The filtered sample was analysed to estimate the content of VFA, percentages of acetic, propionic and butyric acids (by means of a gas chromatographic method), pH

value, ammonia content and numbers of infusoria. The pH value was estimated by means of a potentiometric method. The ammonia content was estimated using Conway's method (AOAC, 1980). Sample preparation and analyses of rumen fluid (including the counting of infusoria numbers) were carried out according to a method described by Hofírek and Dvořák (2002). Numbers of infusoria were counted under a microscope using the Fuchs-Rosenthal chamber.

The obtained values were compared with reference data (Vrzgula et al., 1990). In addition to the parameters of rumen fermentation the composition of feeding ration was also analysed. We determined chemical composition, nutritional value (PDI and energy content were calculated according to Sommer et al., 1994) and ruminal degradability (using the *in vivo* CP method). Silage quality was evaluated as well. Analytical methods of the evaluation of fermentation process were described earlier (Doležal, 2002).

Parameters of rumen fermentation were statistically processed using the analysis of variance and the differences between means were evaluated by the *t*-test. The analysis was performed using the Statgraphic programme, version 5.0.

RESULTS AND DISCUSSION

Fermentation characteristics of silages used in this experiment are presented in Table 1. As far as the course of the fermentation process itself was concerned, the obtained results indicated that maize silage could be considered as a feed of good quality because the final DM content and pH value corresponded to recommended values (Weissbach et al., 1983). The ratio of lactic to acetic acid was relatively low (1.81) similarly like the total content of acids in 1 kg of DM (73.4 g). These results also indicated that in maize silage the course of fermentation was heterogeneous, i.e. the production of lactic acid was reduced. The occurrence of volatile fatty acids in maize silage and haylage from fresh or wilted herbage (grasses, red clover) was described by Kalač (1987). Our observations corroborated data published by Kalač (1987). Although the content of DM was higher than 40% (43.43%) and it belonged to the category of semi-proteinaceous feeds, clover-grass silage showed an increased content of fermentation acids and a very favourable ratio between lactic and acetic acid (5.30). The pH value (4.49) of this silage was also very favourable and contributed to its good stability. An efficient inoculation of ensiled clover-grass stand resulted in a reduced degradation of protein (expressed as the ratio NH_3-N/N total), which was lower than 5%. The total amount of fermentation acids which were ingested by dairy cows in silage and loaded their rumen metabolism is presented in Table 1. It was found out that the total daily amount of consumed silage acids was higher than it is usual (915.2 g). Their molar concentration (11.43 mol) was also higher than recommended by Vojtíšek (1998); according to this author, the optimum concentrations for lactating dairy cows should range from 8 to 9 moles of acids or 1.91 moles per 100 kg of live weight.

Table 1. Mean parameters of the quality of fermentation process of silages and the mole values of concentration fermentations acids from silages in ratio of cows

Silages	DM (%)	pH	TA (mg KOH)	LA (g/kg)	AA (g/kg)	Σ acids (g/kg)	Ethanol (g/kg)	GP (%)	WSC (%)	Quality of fermentation
Maize	33.65	3.89	1 710	15.9	8.8	24.7	0.12	5.87	0	II.
Clover-grass	43.43	4.49	1 350	28.1	5.3	33.4	0.20	4.69	0.12	I.

Quantity of silage/day	LA (g)	AA (g)	Sum of acids (g/cow)	Molar concentration
Maize silage (16 kg)	240	140.8	380.8	5.02
Clover-grass haylage (16 kg)	449.6	84.8	534.4	6.41
Total	689.6	225.6	915.2	11.43

DM – dry matter; TA – titratable acidity; LA – lactic acid; AA – acetic acid; GP – grade of proteolysis (% N-NH_3 of total N); WSC – water soluble saccharides

Table 2. Chemical composition and nutritive values of tested feeds (in DM)

Groups of fodders	CP	Fat	CF	Rumen degradability of CP	NFE	OM	PDIN	PDIE	ME	NEL	NEV	Ca	P
	(g)			(%)	(g)				(MJ)			(g)	
Clover-grass haylage	173.10	39	248.9	77.12	448.9	909.9	98.96	69.99	10.21	6.08	6.01	10.73	2.84
Maize silage	10.42	30	206.0	72.84	612.6	952.8	65.84	72.56	10.78	6.49	6.53	3.65	2.86
Meadow hay	118.10	16	318.8	65.00	463.1	916.0	73.40	76.10	8.30	4.75	4.37	9.00	2.80

CP – crude protein; CF – crude fibre; NFE – N-free extract; OM – organic matter; PDIN, PDIE – really digestible protein in the small intestine; ME – metabolisable energy; NEL – net energy of lactation; NEV – net energy of growth

Chemical composition and nutritive values of silage and hay are presented in Table 2. As one can see, the highest concentration of fibre in 1 kg of DM was found out in meadow hay (318.8 g). According to the fibre content clover-grass silage was made of fodder harvested in an optimum stage of maturity (248.9 g). According to Kováčová (2001), the motoric movements of rumen are inhibited at higher dietary concentrations of fibre and the fraction of rumen non-degradable protein, which is mostly bound to fibre, is not digested in the intestines (similarly like fibre). This results in a reduced intestinal digestibility of protein. In our experiment, the highest ruminal degradability of protein was found out for clover-grass silage (77.12%); this observation was closely associated with a higher protein content and corresponded with data published by other authors (Třináctý et al., 1999).

The results of statistical analyses of the effects of different doses of SC-47 yeast culture on some biochemical parameters of ruminal fermentation are presented in Table 3 and Figures 1–4. These data indicate that the addition of increasing doses of SC-47 yeast culture resulted in significantly different effects of this product on the course of ruminal fermentation. In experimental animals the pH value of rumen fluid was at the lower limit of reference range (Vrzgula et al., 1990) and was statistically significantly lower ($P < 0.01$) than in controls (6.3 ± 0.10). Differences in the pH values of rumen fluid sampled from experimental dairy cows were statistically insignificant. In this experiment it was not possible to corroborate conclusions published by other authors (Lyons, 1993; Kamra et al., 2002; Auclair, 2004 – pers. comm.) that the supplement of yeast products increased and stabilised pH values of rumen fluid. These results corresponded with observations of other authors (Kung et al., 1997; Putnam et al., 1997; Sullivan and Martin, 1999) who did not demonstrate a stabilising effect of yeast culture on pH and some other products of ruminal fermentation. These results were also documented by regression analysis of the relationship between the dependent (pH of rumen fluid) and independent (the dose of yeast culture) variables (Figure 1) which indicated that the increasing dose of yeast culture did not result in a stabilisation of rumen fluid pH value (as compared with the control) ($r = 0.671$).

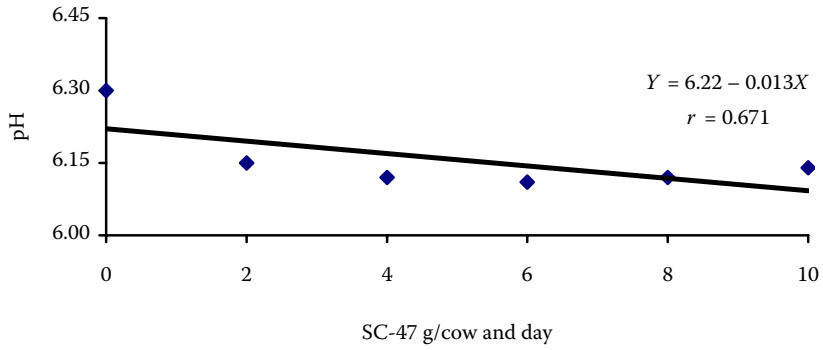

Figure 1. The effect of different levels of SC-47 addition on the pH value of rumen fluid

Table 3. The effect of different levels of yeast culture addition on the parameters of rumen fluid

Parameter		Yeast culture (g/cow/day)					
		0	2	4	6	8	10
pH	M ± SE	6.30 ± 0.10^{BCDEF}	6.15 ± 0.05^{A}	6.12 ± 0.02^{A}	6.11 ± 0.01^{A}	6.12 ± 0.02^{A}	6.14 ± 0.01^{A}
	VC (%)	1.41	0.73	0.29	0.13	0.31	0.20
VFA (mmol/l)	M ± SE	84.0 ± 5.66^{BCDEF}	104.2 ± 2.32^{ACDEF}	111.0 ± 0.89^{ABEFd}	115.8 ± 1.17^{ABFce}	121.0 ± 1.41^{ABCFd}	127.6 ± 1.85^{ABCDE}
	VC (%)	6.73	2.22	0.81	1.01	1.17	1.46
Ammonia (mmol/l)	M ± SE	9.06 ± 0.21^{BCD}	8.12 ± 0.08^{ACDEF}	8.76 ± 0.06^{ABEF}	8.68 ± 0.08^{ABEF}	9.00 ± 0.07^{BCD}	9.00 ± 0.07^{BCD}
	VC (%)	2.04	0.92	0.56	0.86	0.70	0.70
Protozoa (ths/ml)	M ± SE	302.0 ± 12.35^{BCDEF}	337.0 ± 6.21^{ACDEF}	351.20 ± 0.84^{ABEFd}	359.20 ± 1.30^{ABEFc}	372.00 ± 1.58^{ABCDF}	384.80 ± 0.84^{ABCDE}
	VC (%)	3.65	1.65	0.21	0.33	0.38	0.20

M ± SE – mean ± standard error; VC – variation coefficient; VFA – volatile fatty acids
^{A,B,C,D,E,F}significant differences at a significance level of 99% ($P < 0.01$)
^{a,b,c,d,e,f}significant differences at a significance level of 95% ($P < 0.05$)

The effect of the addition of SC-47 yeast culture on the production and content of VFA in the rumen fluid of experimental dairy cows is presented in Table 3 and Figure 2. According to Vrzgula et al. (1990), Bíreš (2000) and other authors the total content of VFA and their percentage contents in rumen change in dependence on both the qualitative and quantitative composition of the diet. The majority of authors reported the values between 80 and 120 mmol/l of rumen fluid as a physiological reference range (Vrzgula et al., 1990). The obtained results indicate that the increasing doses of yeast culture caused a statistically significant ($P < 0.01$) increase in VFA production in experimental animals from 84.0 ± 5.66 mmol/l to as much as 127.6 ± 1.85 mmol/l of rumen fluid. These results correspond with data published by other authors (Lyons, 1993; Brydl et al., 1995, 1998; Doreau and Jouany, 1998; Pestevsek et al., 1998; Sullivan and Martin, 1999; Kamra et al., 2002; and others). On the other hand, in their experiments Putnam et al. (1997), Biricik and Yavuz (2001) and Alshaikh et al. (2002) did not find any marked changes in the production of ruminal VFA after the application of yeast cultures. The regression analysis revealed a very close relationship between the content of VFA and the dose of yeast culture ($r = 0.956$).

The effect of yeast culture supplement on the content of ammonia in the rumen fluid of experimental groups (as compared with controls) is presented in Table 3 and Figure 3. These data indicate that in three groups of experimental dairy cows the addition of yeast product resulted in a statistically higher ($P < 0.01$) utilisation of ammonia than in controls (9.06 ± 0.21 mmol/l). The lowest concentration of ammonia was recorded after the application of 2 g (8.12 ± 0.07 mmol/l) while after the application of 8 and 10 g of yeast culture the observed effect was the same (9.00 ± 0.08 mmol/l). The obtained results corroborate earlier observations published by Lyons (1993), Putnam et al. (1997), Biricik and Yavuz (2001), Alshaikh et al. (2002), Kamra et al. (2002), Nursoy and Baytok (2003) and Strohlein (2003), who did not find any significant effects of the added yeast product on ammonia content in rumen fluid while Newbold et al. (1996) and Sullivan and Martin (1999) demonstrated its increase. As mentioned in many papers, the intensity of bacterial synthesis in rumen was closely correlated with the amount of available energy and also depended on the activity of bacterial microorganisms. The synthesis of 7.5–10.5 g protein required 1.0 MJ

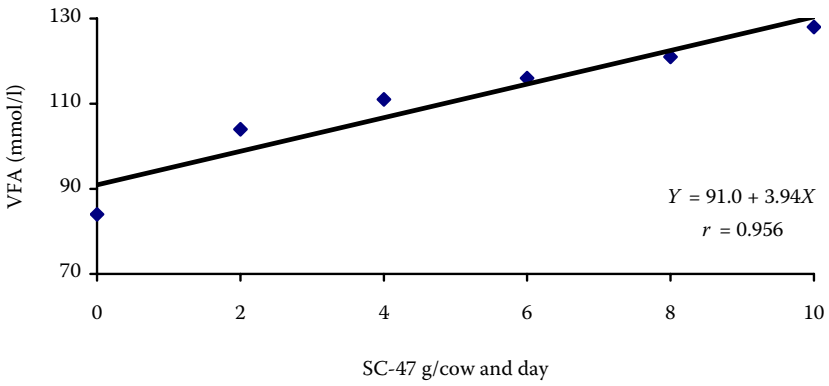

Figure 2. The effect of different levels of SC-47 addition on the VFA content in rumen fluid

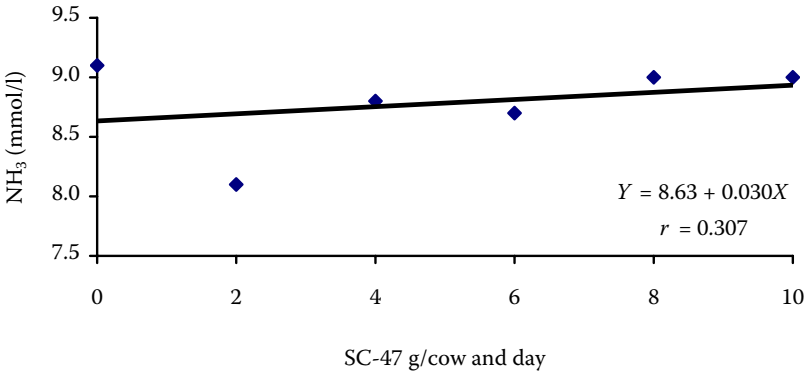

Figure 3. The effect of different levels of SC-47 addition on the NH3 content in rumen fluid

of ME. The lack of available energy resulted in a decrease in the amount of microbial protein and infusoria numbers. It is known that the reduction of protein synthesis in rumen occurs in case that the diet contains a high proportion of concentrates (above 70%) and/or in animals with acute rumen acidosis. A higher content of ammonia in rumen fluid is often associated with a low proportion of roughage in the diet and also with a higher proportion of easily degradable protein (fraction of quickly soluble CP) in rumen (Sommer and Petrikovič, 2003). The analysis of different concentrations of yeast culture revealed that the relationship between the dependent and independent variables was only weak (r = 0.307).

The effect of increasing doses of yeast culture on the average content of rumen infusoria is illustrated in Table 3 and in Figure 4. The obtained results demonstrate a highly significant ($P < 0.01$) relationship between the dose of yeast culture on the one hand and the metabolic activity of infusoria on the other. As compared with controls, the increasing dietary content of yeast culture significantly ($P < 0.01$) stimulated the metabolic activity of rumen infusoria; this was manifested in their significantly increased numbers in the rumen flu-

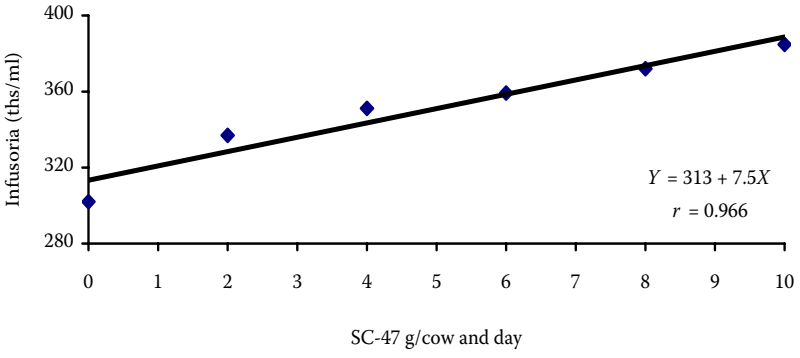

Figure 4. The effect of different levels of SC-47 addition on the ruminal protozoa content

id of experimental animals. The highest number (384.8 ± 0.84 ths/ml) was found out in dairy cows with the dietary supplement of 10 g of yeast culture while in controls the concentration of protozoa in 1 ml of rumen fluid was only 302.0 ± 12.35 ths. In experimental groups receiving 4 and 6 g/head/day a significant ($P < 0.05$) increase in protozoa numbers was found in the group receiving a higher dose of yeast culture. All experimental groups of dairy cows showed a significantly ($P < 0.01$) higher number of ruminal infusoria than controls. Similar results were published also by Lyons (1993), Strzetelski et al. (1996), Yoon and Stern (1996), Alshaikh et al. (2002), Ando et al. (2004), Auclair (2004) and others. On the other hand, Kamra et al. (2002) did not observe any positive effects of yeast supplements on activities of infusoria. It should be said that a decrease in infusoria numbers results in a reduction of microbial protein synthesis. Protozoa are very sensitive above all to changes in pH of rumen fluid and for that reason a decrease of this parameter below the physiological limit causes their quick disappearance from the rumen environment. The obtained results indicate an explicit and statistically highly significant ($P < 0.01$) relationship between the applied dose of yeast culture and infusoria numbers per ml of rumen fluid (Figure 4). The high correlation coefficient ($r = 0.966$) indicates a close relationship between both variables.

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