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BARD Project Number: IS-3988-07

Date of Submission of the report: July 27, 2011

Project Title: Enhancing sustainability of cattle production systems through discovery of biomarkers for feed efficiency

Investigators	Name	Institutions
Principal Investigator (PI)	A. Brosh	Agricultural Research Organization
Co-Principal Investigator (Co-PI)	G. Carstens	Texas Agricultural Experiment Station
Collaborating Investigators:	K. Johnson	Washington State University
	A. Shabtay	Agricultural Research Organization
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Keywords: Beef, Dairy, Energetic Efficiency, Heat Production, Nutrient Requirements.

Abbreviations:

ADG	Average daily gain
BCS	Body condition score
DM	Dry matter
DMI	Dry matter intake (kg/d)
FB	Feeding bouts (events/d)
HP	Heat production (Mcal/d)
ME	Metabolizable energy (Mcal/kg DM)
MEI	Metabolizable energy intake (Mcal/day)
RE	Recovered energy (Mcal/day; milk and body tissue energy)
RFI	Residual feed intake (kg/d; actual DMI minus expected DMI by regression method)
RMEIr	Residual MEI (Mcal/d; actual MEI minus expected MEI by regression method)
RMEIc	Residual MEI (Mcal/d; actual MEI minus expected MEI calculated by NRC equations)
RHP	Residual HP (Mcal/d; actual HP minus expected HP calculated by NRC equations)
WOL	Week of lactation

Budget: IS: \$161,462 US: \$191,756 Total: \$353,218

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

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Publication Summary:

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged	0	2	0	2
Submitted, in review, in preparation	1	1	0	2
Invited review papers	0	0	0	0
Book chapters	0	0	0	0
Books	0	0	0	0
Master theses	0	1	1	2
Ph.D. theses	0	1	1	2
Abstracts	0	7	5	12
Non-Refereed (proceedings, reports, etc.)	1	3	0	4

Postdoctoral Training: none

Cooperation Summary:

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	0	1	4	5
Longer Visits (Sabbaticals)	0	0	0	0

Description Cooperation:

Dr. Brosh (Israeli PI, Newe Yaar Research Center) traveled to Texas Agricultural Experiment Station (Texas A&M University) in 2007 to plan for the project, review facilities, and establish research protocols for the project studies. Dr. Brosh, Dr. Carstens (US Co-PI, Texas A&M University) and Dr. Johnson (US Collaborator, Washington State University) formally met to present project updates and review upcoming project activities during the annual national meetings of the American Societies of Animal Science and Dairy Science Associations in 2007 and 2009. Additionally, Drs. Brosh, Carstens and Johnson met to discuss project activities during the International Symposia on Energy and Protein Metabolism and Nutrition in Clermont-Ferrand, France (2007) and Parma, Italy (2010).

Patent Summary:

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted	0	0	0	0
Issued (allowed)	0	0	0	0
Licensed	0	0	0	0

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ABSTRACT

Feed inputs represent the largest variable cost of producing meat and milk from ruminant animals. Thus, strategies that improve the efficiency of feed utilization are needed to improve the global competitiveness of Israeli and U.S. cattle industries, and mitigate their environmental impact through reductions in nutrient excretions and greenhouse gas emissions. Implementation of innovative technologies that will enhance genetic merit for feed efficiency is arguably one of the most cost-effective strategies to meet future demands for animal-protein foods in an environmentally sustainable manner. While considerable genetic variation in feed efficiency exist within cattle populations, the expense of measuring individual-animal feed intake has precluded implementation of selection programs that target this trait. Residual feed intake (**RFI**) is a trait that quantifies between-animal variation in feed intake beyond that expected to meet energy requirements for maintenance and production, with efficient animals being those that eat less than expected for a given size and level of production. There remains a critical need to understand the biological drivers for genetic variation in RFI to facilitate development of effective selection programs in the future.

Therefore, the aim of this project was to determine the biological basis for phenotypic variation in RFI of growing and lactating cattle, and discover metabolic biomarkers of RFI for early and more cost-effective selection of cattle for feed efficiency. Objectives were to: (1) Characterize the phenotypic relationships between RFI and production traits (growth or lactation), (2) Quantify inter-animal variation in residual HP, (3) Determine if divergent RFI phenotypes differ in HP, residual HP, recovered energy and digestibility, and (4) Determine if divergent RFI phenotypes differ in physical activity, feeding behavior traits, serum hormones and metabolites and hepatic mitochondrial traits.

The major research findings from this project to date include:

In lactating dairy cattle, substantial phenotypic variation in RFI was demonstrated as cows classified as having low RMEI consumed 17% less MEI than high-RMEI cows despite having similar body size and lactation productivity. Further, between-animal variation in RMEI was found to moderately associated with differences in RHP demonstrating that maintenance energy requirements contribute to observed differences in RFI. Quantifying energetic efficiency of dairy cows using RHP revealed that substantial changes occur as week of lactation advances—thus it will be critical to measure RMEI at a standardized stage of lactation. Finally, to determine RMEI in lactating dairy cows, individual DMI and production data should be collected for a minimum of 6 wk.

We demonstrated that a favorably association exists between RFI in growing heifers and efficiency of forage utilization in pregnant cows. Therefore, results indicate that female progeny from parents selected for low RFI during postweaning development will also be efficient as mature females, which has positive implications for both dairy and beef cattle industries. Results from the beef cattle studies further extend our knowledge regarding the biological drivers of phenotypic variation in RFI of growing animals, and demonstrate that significant differences in feeding behavioral patterns, digestibility and heart rate exist between animals with divergent RFI. Feeding behavior traits may be an effective biomarker trait for RFI in beef and dairy cattle.

There are differences in mitochondrial acceptor control and respiratory control ratios between calves with divergent RFI suggesting that variation in mitochondrial metabolism may be visible at the genome level. Multiple genes associated with mitochondrial energy processes are altered by RFI phenotype and some of these genes are associated with mitochondrial energy expenditure and major cellular pathways involved in regulation of immune responses and energy metabolism.

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ACHIEVEMENTS

Lactating Dairy Cattle Studies: To determine if between-animal variation in heat production contributes to observed phenotypic differences in RFI of lactating dairy cows, individual DMI, milk energy, BW and BCS were measured in Holstein cows (N = 40) over 3 lactation periods from 16 to 30 WOL. Residual MEI (**RMEIr**) was calculated weekly as the difference between actual and expected MEI from linear regression of MEI on mid-test $BW^{0.75}$ and RE (milk and tissue energy). From 16 to 30 WOL, cows with low RMEIr (< 0.50 SD from mean) consumed 17% less ($P < 0.001$) MEI (59.7 vs 70.0 ± 1.6 Mcal/d) than high-RMEIr cows (> 0.50 SD from mean) despite have similar BW (648 vs 627 ± 15 kg) and milk energy (31.2 vs 30.6 ± 0.8 Mcal/d). Body condition scores during 16 to 30 WOL were higher in cows with low RMEIr (2.56 vs 2.44 ± 0.03) compared to cows with high RMEIr. Heat production was measured during 5-d periods 2 to 3 times from 16 to 30 WOL using the O_2 pulse-heart rate method. Residual HP (**RHP**) was calculated as the difference between actual HP and expected HP based on NRC equations. Cows with low RMEIr tended ($P = 0.07$) to have lesser RHP (-2.14 vs 0.04 ± 0.82 Mcal/d) than high-RMEIr cows. Physical activity (step counts/d) was numerically lower in cows with low RMEIr, but was not significantly different from high-RMEIr cows. Therefore, substantial phenotypic variation in RFI exists in lactating dairy cows, and that between-animal variation in RFI of lactating cows is related to differences in HP.

Time-series changes in energetic efficiency of lactating cows was examined using energy balance data measured in cows (N = 73) over 3 lactation periods from 1 to 40 WOL. As expected, RE/MEI was positively correlated with milk production throughout lactation. However, RMEIr and RHP were both negative during early lactation, such that cows consumed less MEI and produced less heat than expected. RHP increased to zero by the 13th WOL and remained relatively constant from 13 to 40 WOL. Likewise, RMEI increased to 6.3 Mcal/d by the 16th WOL and averaged 7.7 Mcal/d from 16 to 40 WOL. Improved energetic efficiency during early lactation is associated with lower maintenance energy requirements relative to BW. The increase in RHP that was observed during the first 8 WOL coincides with the proportional growth in visceral organ tissues associated with early-lactation increases in DMI.

To determine the minimal length of time required to measure RMEIr in dairy cows, weekly energy balance data from 40 cows during 16 to 30 WOL were analyzed. RMEIr was summarized for first 14 d (16 to 17 WOL), and the first 21, 28, 35, 42, 49 and 56 d (16 to 23 WOL), and compared to RMEIr measured during the entire 105 d period (16 to 30 WOL). There was 50% total agreement in ranking cows into low, medium and high RMEIr phenotype groups (average $\pm$

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0.50 SD) based on using only the first 14- or 21-d RMEIr. The total agreement increased to 60, 62, 68, 70, 80% when RMEIr data for the first 28, 35, 42, 49 and 56 d, respectively, was considered. Individual DMI and performance traits should be measured for a minimum of 6 wk to determine RMEIr in lactating dairy cows.

Beef Cattle Studies: To determine if between-animal variation in nutrient digestibility contributes to observed phenotypic differences in RFI, DMI and performance traits were measured in Brangus heifers for 3 yr (N = 116/yr). Heifers were ranked by RFI each yr and those with the lowest (n = 20/yr) and highest (n = 20/yr) RFI used to determine diet DM and nutrient digestibilities with acid-insoluble ash used as an internal marker. Heifers with low RFI consumed 18% less DMI (8.76 vs 10.69 ± 0.21 kg/d) despite having similar BW and ADG. Heifers with low RFI had 6% higher ($P < 0.001$) apparent diet DM (790 vs 745 ± 38 g/kg DM), and neutral detergent fiber, crude protein and phosphorus digestibilities compared to high-RFI heifers. Heifers with low RFI had fecal nitrogen and phosphorus excretion rates that were 36 and 32% lower, respectively, than in high-RFI heifers. Results suggest that between-animal variation in RFI was related to differences in apparent nutrient digestibility.

To determine if between-animal variation in feeding behavior activities contributes to observed differences in RFI, feeding behavior traits and DMI were measured in 340 Angus steers fed a high-grain diet (ME = 3.1 Mcal/kg DM). Steers with low-RFI consumed 15% less DMI, and had fewer ($P < 0.01$) feeding bouts (**FB**) and shorter daily FB durations (53.1 vs 65.4 ± 1.2 min/d) than high-RFI steers. Meal criterion (29.0 vs 24.9 ± 0.03 min) was longer ($P < 0.05$) for low-RFI steers indicating they took more time to initiate their next meal. Daily meal duration (122 vs 140 ± 4 min/d) was less for steers with low RFI compared to high-RFI steers. Steers with divergent RFI had distinctive feeding behavior patterns, which suggests that feeding behavior traits may be an effective biomarker for RFI in beef cattle.

To determine if RFI measured in growing heifers was associated with subsequent efficiency of forage utilization during the 2nd trimester of gestation, RFI was measured in Bonsmara heifers for 2 years (N = 57/yr). Heifers with the lowest (n = 12/yr) and highest (n = 12/yr) RFI were retained for breeding. As heifers, those with low RFI consumed 20% less DMI (7.28 vs 9.07 ± 0.23 kg/d), but had similar BW and ADG compared to high-RFI heifers. First and 2nd parity females from trials 1 and 2 were subsequently fed a hay diet (ME = 2.11 Mcal/kg DM), and DMI, feeding behavior and heart rate (**HR**) measured. Pregnant females with low RFI as heifers continued to consume less ($P < 0.01$) DMI (9.00 vs 11.62 ± 0.55 kg/d) than high-RFI females even though BW, ADG and BCS were similar. Low-RFI females had lesser ($P < 0.05$) daily FB

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durations (149 vs 198 ± 13 min/d) and HR (65.8 vs 71.1 ± 1.7 beats/min) during mid-gestation then high-RFI females. Females with low RFI as heifers consumed 23% less forage during mid-gestation than high-RFI females, demonstrating that RFI of growing heifers was favorably associated with subsequent cow efficiency. Results suggest that between-animal variation in RFI was related to differences in HR and feeding behavior patterns.

We hypothesized that variation in RFI was associated with hepatic mitochondrial metabolism and associated genes expression. Hepatic mitochondrial metabolism was examined in 2 populations (Bonsmara heifers and Angus steers) with divergent RFI phenotypes. Differences in mitochondrial acceptor control and respiratory control ratios between calves with divergent RFI suggested that variation in mitochondrial metabolism may be evident at the genome level. Liver samples from these same animals provided RNA for a bovine microarray analysis using a customized array that included 13 mitochondrial genes. Four mitochondrial genes were differentially expressed in heifers with divergent RFI phenotypes. NADP-dependent malic enzyme, ATP synthase, and 18 kDa mitochondrial protein were more highly expressed ($P < 0.05$) in the inefficient phenotypes, whereas, L53 39S ribosomal protein was more highly expressed in the efficient phenotypes. Four additional mitochondrial genes tended ($P < 0.10$) to be differentially expressed between heifer RFI phenotypes; TP-53-induced glycolysis and apoptosis regulator, mitochondrion associated apoptosis-inducing factor, methionyl-tRNA formyltransferase, and L48 39S ribosomal protein. The mitochondrial genes TP-53-induced glycolysis and apoptosis regulator and cytochrome P450 11A1 had greater expression ($P < 0.05$) and monofunctional C1-tetrahydrofolate synthase tended ($P < 0.10$) to have greater expression in the efficient steer phenotypes. The absence of common differentially expressed genes for these 2 populations suggests that gender and (or) diet may have influenced expression of genes associated with RFI phenotypes. These results demonstrate that genes associated with mitochondrial energy processes are altered by RFI phenotype.

A pathway analysis of the microarray data was conducted to determine if alterations in metabolic pathways might reveal large physiological changes when examined collectively to provide insight into differences between RFI phenotypes. Results suggest that inflammatory response genes (SIRT1; gene in energy expenditure pathways) and JAK/STAT signaling pathway genes (development and homeostasis) may be possible candidate genes for future research. Multiple genes are responsible for animal variation in RFI, and that some of these genes are associated with mitochondrial energy expenditure and major cellular pathways involved in regulation of immune responses and energy metabolism.

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DETAILS OF COOPERATION

The aim of this multidisciplinary project was to develop new technologies to improve feed efficiency of growing and lactating cattle. While considerable genetic variation in feed efficiency exist within cattle populations, the expense of measuring feed intake has precluded implementation of selection programs for this trait in beef and dairy cattle. This project has enabled characterization of feed efficiency traits (e.g., RFI) across a continuum of animal types, and to examine energetic efficiency on a whole-animal to subcellular basis. The Israeli group was able to extend the RFI concept to lactating dairy cattle, and demonstrate that phenotypic variation in this trait is linked to heat production. This to our knowledge is a novel finding, and was accomplished as a result of the combined expertise of our group. Moreover, our research has demonstrated that RFI in developing beef heifers was favorably associated with efficiency of forage utilization in pregnant cows. These research findings have positive implications for the dairy and beef industries in Israel and the US as these industries strive to become more economically and environmentally sustainable. The US group, with substantial input from Israeli scientists, was further able to extend knowledge regarding the biological drivers of phenotypic variation in RFI of growing cattle, demonstrating that significant differences in feeding behavioral patterns, digestibility and hepatic gene expression exist between animals with divergent RFI. Information from this research will allow the beef and dairy industries to develop proxy traits to enhance selection of animals with favorable genotypes for feed efficiency. Furthermore, the gene expression research findings provide foundational insight into the energetic basis for phenotypic variation in RFI, and will generate future opportunities for exploration of targeted metabolic pathways. This project has successfully linked expertise from the collaborating laboratories, and the results generated from this project will yield substantial benefits for the cattle industries in both countries. Finally, results from this project have generated a number of follow-up hypotheses that will extend fruitful collaboration into the future.

LIST OF PUBLICATIONS

- Bottje, W.G. and G.E. Carstens. 2009. Association of mitochondrial function and feed efficiency in poultry and livestock species. *J. Anim. Sci.* 87:E48-63E.
- Mendes, E.D.M, G.E. Carstens, L.O. Tedeschi, Pinchak, W.E. and T.H. Friend. 2011. Validation of a system for monitoring feeding behavior in beef cattle. *J. Anim. Sci.* doi:10.2527/jas.2010-3489.
- Krueger, W.K., G.E. Carstens, R.R. Gomez, B.M. Bourg, P.A. Lancaster, L.J. Slay, J.C. Miller, R.C. Anderson, S.M. Horrocks, N.A. Krueger, and T.D.A. Forbes. 2011. Relationships between residual feed intake and apparent nutrient digestibility, in vitro methane producing activity and VFA concentrations in growing Brangus heifers. *J. Anim. Sci.* (Submitted).

APPENDIX

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Referred Journal Publications

Published

Bottje, W.G. and G.E. Carstens. 2009. Association of mitochondrial function and feed efficiency in poultry and livestock species. *J. Anim. Sci.* 87:E48-63E.

Mendes, E.D.M, G.E. Carstens, L.O. Tedeschi, Pinchak, W.E. and T.H. Friend. 2011. Validation of a system for monitoring feeding behavior in beef cattle. *J. Anim. Sci.* doi:10.2527/jas.2010-3489.

Submitted

Krueger, W.K., G.E. Carstens, R.R. Gomez, B.M. Bourg, P.A. Lancaster, L.J. Slay, J.C. Miller, R.C. Anderson, S.M. Horrocks, N.A. Krueger, and T.D.A. Forbes. 2011. Relationships between residual feed intake and apparent nutrient digestibility, in vitro methane producing activity and VFA concentrations in growing Brangus heifers. *J. Anim. Sci.* (Submitted).

Student Thesis/Dissertation

Mendez, E.D.M. Characterization of feeding behavior traits and associations with performance and feed efficiency in finishing steers. M.S. Thesis, Texas A&M University, August 2010.

Asher, A. The influence of week of lactation on the energy expenditure and the efficiency characters in Holstein cows. M.S. Thesis, The Hebrew University of Jerusalem, Approved October 2010.

Krueger, W.K. Understanding beef cattle efficiency: Physiological and digestive factors affecting residual feed intake and effects of tannin supplementation on animal performance, fermentation and carcass traits. Ph.D. Dissertation. Texas A&M University. August 2009.

Asher, A. Identification of individual efficiency characters in Holstein cows and bulls and the effect of photoperiod on production efficiency. Ph.D. Dissertation. Haifa University. Starting January 2011.

Research Abstracts

Asher, A., J. Miron, G. Adin, U. Mualem, E. Zenou, A. Shabtay, A. Arieli, I. Halachmi, Y. Aharoni and A. Brosh. 2008. Production efficiency of lactated dairy cows: comparison of tables calculated vs. measured in-vivo ME concentration values. The 20th Annual Meeting of Cattle Sciences. Pp. 131-132 (Abstr.).

Asher, A., J. Miron, G. Adin, A. Shabta, A. Zenou, M. Nikbachat, S. Yaacobi, Y. Portnik, U. Moualem, A. Arieli, Y. Aharoni and A. Brosh. 2010. The influence of week of lactation on the energy expenditure and the efficiency characters in Holstein cows. The 22th Annual Meeting of Cattle Sciences, Pp. 73-74 (Abstr.).

Asher, A., A. Haim, A. Shabtay, J. Miron, G. Adin, I. Halachmi, A. Zenou, M. Nikbachat, S. Yaacobi, U. Moualem, A. Arieli, Y. Aharoni and A. Brosh. 2011. Identification of production efficiency in Holstein cows and the effect of photoperiod on production efficiency. The 23th Annual Meeting of Cattle Sciences. Pp. 68-69 (Abstr.).

Bailey, J.C., G.E. Carstens, J.T. Walter, A.N. Hafla, E.D. Mendes, L.O. Tedeschi and R.K. Miller. 2011. Effects of residual feed intake classification and breed type on feed efficiency and feeding behavior traits in heifers fed a high-grain diet. *J. Anim. Sci.* 89(E-Suppl. 1):366.

Bailey, J.C., L.O. Tedeschi, E.D. Mendes and G.E. Carstens. 2011. Evaluation of bimodal distributions to determine meal criterion in heifers fed a high-grain diet. *J. Anim. Sci.* 89(E-Suppl. 1):761.

Brosh, A., A. Asher, J. Miron, A. Shabtay, G. Adin, U. Moallem, Y. Aharoni A. Arieli. 2009. Residual feed intake and heat production of Holstein cows throughout lactation. J. Anim. Sci. 87(E-Suppl. 1):533.

Brosh, A. Asher, A., Miron, J., Adin, G., Moualem, U., Shabtay, A., Zenou, A., Nikbachat, M., Yaacobi, S., Portnik, Y., Halchmi, I., Arieli, A., Aharoni Y.(2010). Production efficiency of the Israeli Holstein cow, the effects of photoperiod and milk production. The 22th Annual Meeting of Cattle Sciences, May 04-06, 2010 Renaissance Hotel, Jerusalem, Israel, pp. 75-76. Abstract and oral presentation.

Hafla, A.N., G.E. Carstens, T.D.A. Forbes, J.C. Bailey, J.T. Walter, J.W. Holloway and J.G. Moreno. 2011. Relationship between postweaning RFI in heifers and intake and productivity of mid-gestation beef females. J. Anim. Sci. 89(E-Suppl. 1):416.

Krueger, W.K., G.E. Carstens, R.R. Gomez, B.M. Bourg, P.A. Lancaster, L.J. Slay, J.C. Miller, R.C. Anderson, S.M. Horrocks, N.A. Krueger, and T.D.A. Forbes. 2009. Relationships between residual feed intake and apparent nutrient digestibility, in vitro methane producing activity and VFA concentrations in growing Brangus heifers. J. Anim. Sci. 87(E-Suppl. 2):153.

Lancaster, P.A., G.E. Carstens, P. Chen, D.W. Forrest, T.H. Welsh, Jr., R.D. Randel and T.D.A. Forbes. 2008. Relationship between residual feed intake and reproductive performance in Brangus heifers. J. Anim. Sci. 86(E-Suppl. 2):199.

Mendes, E.D.M., G.E. Carstens and L. Tedeschi. 2010. Evaluation of feed behavior traits in beef heifers using a GrowSafe intake measurement system. J. Anim. Sci. 88(E-Suppl. 2):18.

Mendes, E.D.M., G.E. Carstens and L. Tedeschi. 2010. Characterization of feeding behavior traits and associations with feed efficiency in beef heifers fed a high-grain diet. J. Anim. Sci. 88(E-Suppl. 2):791.

Non-referred Publications

Carstens, G.E., P.A. Lancaster and L.O. Tedeschi. 2008. Bringing feed efficiency technologies to the beef industry. Proc. 19th Florida Ruminant Nutrition Symposium, Gainesville, FL. Pp 156-167.

Carstens, G.E. and M.S. Kerley. 2009. Biological basis for variation in energetic efficiency of beef cattle. Proc. 41th Beef Improvement Federation Research Symposium, Sacramento, CA. Pp 124-130.

Carstens, G.E. 2010. What is the biological basis for variation in energetic efficiency of beef cattle? Proc. 14th Colorado Nutrition Roundtable, Fort Collins, CO. Pp 8-14.

Johnson, K.A., H. Neibergs, J.J. Michal, G.E. Carstens, M. Settles, A. Hafla, T.D.A. Forbes, J.W. Holloway and A. Brosh. 2010. Differential expression of mitochondrial genes in liver from beef calves with divergent phenotypes for feed efficiency. In: G. Matteo Crovetto (Ed.), Energy and Protein Metabolism and Nutrition. EAAP Pub. 127:75-76.